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(21) International Application Number: PCT/US99/26039 (22) International Filing Date: 4 November 1999 (04.11.99) (30) Priority Data: 60/107,243 5 November 1998 (05.11.98) US (71) Applicant: EDEN BIOSCIENCE CORPORATION [US/US]; 11816 North Creek Parkway N., Bothell, WA 98011-8205 (US). (72) Inventors: WEI, Zhong-Min; 8230 125th Court, Kirkland, WA 98034 (US). SCHADING, Richard, L.; 3607 Carriage Gate Drive, West Melbourne, FL 32904 (US). (74) Agents: GOLDMAN, Michael, L. et al.; Nixon Peabody LLP, Clinton Square, P.O. Box 1051, Rochester, NY 14603 (US).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> (88) Date of publication of the international search report: 14 September 2000 (14.09.00)
(54) Title: HYPERSENSITIVE RESPONSE ELICITOR-INDUCED STRESS RESISTANCE (57) Abstract <p>The present invention is directed to imparting stress resistance to plants. This can be achieved by applying a hypersensitive response elicitor in a non-infectious form to plants or plant seeds under conditions effective to impart stress resistance to plants or plants grown from the plant seeds. Alternatively, transgenic plants or plant seeds transformed with a DNA molecule encoding the elicitor can be provided and the transgenic plants or plants resulting from the transgenic plant seeds are grown under conditions effective to impart stress resistance to plants or plants grown from the plant seeds.</p>		

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HYPERSENSITIVE RESPONSE ELICITOR-INDUCED STRESS RESISTANCE

5

FIELD OF THE INVENTION

10 The present invention relates to imparting stress resistance to plants with a hypersensitive response elicitor.

BACKGROUND OF THE INVENTION

15 Under both natural and agricultural conditions, plants are exposed to various forms of environmental stress. Stress is mainly measured with respect to growth (i.e. biomass accumulation) or with respect to the primary assimilation processes (i.e. carbon dioxide and mineral intake). Soil water deficits, suboptimal and supraoptimal temperatures, salinity, and poor aeration of soils may each cause some
20 growth restrictions during the growing season, so that the yield of plants at the end of the season expresses only a small fraction of their genetic potential. Indeed, it is estimated that in the United States the yield of field-grown crops is only 22% of genetic potential. The same physicochemical factors can become extreme in some habitats, such as deserts or marshes, and only specially adapted vegetation can
25 complete its life cycle in the unusually hostile conditions. In less extreme environments, individual plants can become acclimated to changes in water potential, temperature, salinity, and oxygen deficiency so that their fitness for those environments improves. Some species are better able to adapt than others, and various anatomical, structural, and biochemical mechanisms account for acclimation.

30 Under natural and agriculture conditions, plants must constantly endure stress. Some environmental factors can become stressful in a very short period of time (e.g., high or low temperature) or may take long periods of time to stress plants (e.g., soil water content or mineral nutrients). Generally, environmental stress effecting plants can be in the form of climate related stress, air pollution stress,

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chemical stress, and nutritional stress. Examples of climate related stress include drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light. Air pollution stress can be in the form of carbon dioxide, carbon monoxide, sulfur dioxide, NO_x, hydrocarbons, ozone, ultraviolet radiation, and acidic rain. Chemical stress can result from application of insecticides, fungicides, herbicides, and heavy metals. Nutritional stress can be caused by fertilizers, micronutrients, and macronutrients.

For most plants, water is essential for growth. Some plants are able to preserve some water in the soil for later use, while others complete their life cycles during a wet season before the onset of any drought. Other plants are able to aggressively consume water to save themselves while causing water deprivation for other plants in that location. Plants lacking any of these capabilities are severely hampered by the absence of water.

Chilling injury occurs in sensitive species at temperatures that are too low for normal growth but not sufficiently low to form ice. Such injury typically occurs in species of tropical or subtropical origin. When chilling occurs, discoloration or lesions appear on leaves giving them a water-soaked appearance. If roots are chilled, the plants may wilt. On the other hand, freezing temperatures and the accompanying formation of ice crystals in plants can be lethal if ice crystals extend into protoplasts or remain for long periods.

Stress is also caused by the other temperature extremes with few plants being able to survive high temperatures. When higher plant cells or tissues are dehydrated or are not growing, they can survive higher temperatures than cells which are hydrated, vegetative, and growing. Tissues which are actively growing can rarely survive at temperatures above 45°C.

High salt concentrations are another form of environmental stress which can afflict plants. In natural conditions, such high concentrations of salt are found close to seashores and estuaries. Farther inland, natural salt may seep from geological deposits adjoining agricultural areas. In addition, salt can accumulate in irrigation water when pure water is evaporated or transpired from soil. About all irrigated farmland is effected by high salt concentrations. High salt content not

only injures plants but degrades soil structure by decreasing porosity and water permeability.

Air pollution in the form of ozone, carbon dioxide, carbon monoxide, sulfur dioxide, NO_x, and hydrocarbons can very adversely effect plant growth by creating smog and environmental warming.

The present invention is directed to overcoming various forms of environmental stress and imparting resistance in plants to such stress.

SUMMARY OF THE INVENTION

The present invention relates to the use of a hypersensitive response elicitor protein or polypeptide to impart stress resistance to plants. In one embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide is applied to plants or plant seeds under conditions effective to impart stress resistance. Alternatively, stress resistance is imparted by providing a transgenic plant or plant seed transformed with a DNA molecule which encodes for a hypersensitive response elicitor protein or polypeptide and growing the transgenic plant or plants produced from the transgenic plant seeds under conditions effective to impart stress resistance.

Stress encompasses any environmental factor having an adverse effect on plant physiology and development. Examples of such environmental stress include climate-related stress (e.g., drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light), air pollution stress (e.g., carbon dioxide, carbon monoxide, sulfur dioxide, NO_x, hydrocarbons, ozone, ultraviolet radiation, acidic rain), chemical (e.g., insecticides, fungicides, herbicides, heavy metals), and nutritional stress (e.g., fertilizer, micronutrients, macronutrients). Applicants have found that use of hypersensitive response elicitors in accordance with the present invention impart resistance to plants against such forms of environmental stress.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the use of a hypersensitive response elicitor protein or polypeptide to impart stress resistance to plants. In one

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embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide is applied to plants or plant seeds under conditions effective to impart stress resistance. Alternatively, the stress resistance is imparted by providing a transgenic plant or plant seed transformed with a DNA molecule which encodes for a hypersensitive response elicitor protein or polypeptide and growing the transgenic plant or plants produced from the transgenic plant seeds under conditions effective to impart stress resistance.

The hypersensitive response elicitor polypeptides or proteins according to the present invention are derived from hypersensitive response elicitor polypeptides or proteins of a wide variety of fungal and bacterial pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor. Examples of suitable bacterial sources of polypeptide or protein elicitors include *Erwinia*, *Pseudomonas*, and *Xanthomonas* species (e.g., the following bacteria: *Erwinia amylovora*, *Erwinia chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas solanacearum*, *Xanthomonas campestris*, and mixtures thereof). In addition to hypersensitive response elicitors from these Gram negative bacteria, it is possible to use elicitors from Gram positive bacteria. One example is *Clavibacter michiganensis* subsp. *sepedonicus*.

An example of a fungal source of a hypersensitive response elicitor protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora*.

The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

	Met	Gln	Ile	Thr	Ile	Lys	Ala	His	Ile	Gly	Gly	Asp	Leu	Gly	Val	Ser
	1				5				10				15			
	Gly	Leu	Gly	Ala	Gln	Gly	Leu	Lys	Gly	Leu	Asn	Ser	Ala	Ala	Ser	Ser
30				20					25				30			
	Leu	Gly	Ser	Ser	Val	Asp	Lys	Leu	Ser	Ser	Thr	Ile	Asp	Lys	Leu	Thr
				35				40					45			
	Ser	Ala	Leu	Thr	Ser	Met	Met	Phe	Gly	Gly	Ala	Leu	Ala	Gln	Gly	Leu
				50			55					60				

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	Gly	Ala	Ser	Ser	Lys	Gly	Leu	Gly	Met	Ser	Asn	Gln	Leu	Gly	Gln	Ser	65	70	75	80
	Phe	Gly	Asn	Gly	Ala	Gln	Gly	Ala	Ser	Asn	Leu	Leu	Ser	Val	Pro	Lys	85	90	95	
5	Ser	Gly	Gly	Asp	Ala	Leu	Ser	Lys	Met	Phe	Asp	Lys	Ala	Leu	Asp	Asp	100	105	110	
	Leu	Leu	Gly	His	Asp	Thr	Val	Thr	Lys	Leu	Thr	Asn	Gln	Ser	Asn	Gln	115	120	125	
10	Leu	Ala	Asn	Ser	Met	Leu	Asn	Ala	Ser	Gln	Met	Thr	Gln	Gly	Asn	Met	130	135	140	
	Asn	Ala	Phe	Gly	Ser	Gly	Val	Asn	Asn	Ala	Leu	Ser	Ser	Ile	Leu	Gly	145	150	155	160
	Asn	Gly	Leu	Gly	Gln	Ser	Met	Ser	Gly	Phe	Ser	Gln	Pro	Ser	Leu	Gly	165	170	175	
15	Ala	Gly	Gly	Leu	Gln	Gly	Leu	Ser	Gly	Ala	Gly	Ala	Phe	Asn	Gln	Leu	180	185	190	
	Gly	Asn	Ala	Ile	Gly	Met	Gly	Val	Gly	Gln	Asn	Ala	Ala	Leu	Ser	Ala	195	200	205	
20	Leu	Ser	Asn	Val	Ser	Thr	His	Val	Asp	Gly	Asn	Asn	Arg	His	Phe	Val	210	215	220	
	Asp	Lys	Glu	Asp	Arg	Gly	Met	Ala	Lys	Glu	Ile	Gly	Gln	Phe	Met	Asp	225	230	235	240
	Gln	Tyr	Pro	Glu	Ile	Phe	Gly	Lys	Pro	Glu	Tyr	Gln	Lys	Asp	Gly	Trp	245	250	255	
25	Ser	Ser	Pro	Lys	Thr	Asp	Asp	Lys	Ser	Trp	Ala	Lys	Ala	Leu	Ser	Lys	260	265	270	
	Pro	Asp	Asp	Asp	Gly	Met	Thr	Gly	Ala	Ser	Met	Asp	Lys	Phe	Arg	Gln	275	280	285	
30	Ala	Met	Gly	Met	Ile	Lys	Ser	Ala	Val	Ala	Gly	Asp	Thr	Gly	Asn	Thr	290	295	300	
	Asn	Leu	Asn	Leu	Arg	Gly	Ala	Gly	Gly	Ala	Ser	Leu	Gly	Ile	Asp	Ala	305	310	315	320
	Ala	Val	Val	Gly	Asp	Lys	Ile	Ala	Asn	Met	Ser	Leu	Gly	Lys	Leu	Ala	325	330	335	
35	Asn	Ala																		

This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains

substantially no cysteine. The *Erwinia chrysanthemi* hypersensitive response elicitor polypeptide or protein is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 2 as follows:

5	CGATTTTACC CGGGTGAACG TGCTATGACC GACAGCATCA CGGTATTCTGA CACCGTTACG	60
	GCGTTTATGG CCGCGATGAA CCGGCATCAG GCGGCGCGCT GGTCCGCCGA ATCCGGCGTC	120
	GATCTGGTAT TTCAGTTTGG GGACACCGGG CGTGAACTCA TGATGCAGAT TCAGCCGGGG	180
	CAGCAATATC CCGGCATGTT GCGCAGCTG CTCGCTCGTC GTTATCAGCA GCGGCGCAGAG	240
	TGCGATGGCT GCCATCTGTG CCTGAACGGC AGCGATGTAT TGATCCTCTG GTGGCCGCTG	300
10	CCGTCGGATC CCGGCAGTTA TCCGCAGGTG ATCGAACGTT TGTTTGAACT GCGGGAATG	360
	ACGTTGCCGT CGCTATCCAT AGCACCGACG GCGCGTCCGC AGACAGGGAA CCGACGCGCC	420
	CGATCATTAA GATAAAGGCG GCTTTTTTTA TTGCAAAACG GTAACGGTGA GGAACCGTTT	480
	CACCGTCGGC GTCACCTCAGT AACAAGTATC CATCATGATG CCTACATCGG GATCGGCGTG	540
	GGCATCCGTT GCAGATACTT TTGCGAACAC CTGACATGAA TGAGGAAACG AAATTATGCA	600
15	AATTACGATC AAAGCGCACA TCGGCGGTGA TTTGGGCGTC TCCGGTCTGG GGCTGGGTGC	660
	TCAGGGACTG AAAGGACTGA ATTCCGCGGC TTCATCGCTG GGTCCAGCG TGGATAAACT	720
	GAGCAGCACC ATCGATAAGT TGACCTCCGC GCTGACTTCG ATGATGTTTG GCGGCGCGCT	780
	GGCGCAGGGG CTGGGCGCCA GCTCGAAGGG GCTGGGGATG AGCAATCAAC TGGGCCAGTC	840
	TTTCGGCAAT GGCGCGCAGG GTGCGAGCAA CTTGCTATCC GTACCGAAAT CCGGCGGCGA	900
20	TGCGTTGTCA AAAATGTTTG ATAAAGCGCT GGACGATCTG CTGGGTCATG ACACCGTGAC	960
	CAAGCTGACT AACCAGAGCA ACCAACTGGC TAATTCAATG CTGAACGCCA GCCAGATGAC	1020
	CCAGGGTAAT ATGAATGCGT TCGGCAGCGG TGTGAACAAC GCACTGTCGT CCATTCTCGG	1080
	CAACGGTCTC GGCCAGTCGA TGAGTGGCTT CTCTCAGCCT TCTCTGGGGG CAGGCGGCTT	1140
	GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGGCGT	1200
25	GGGGCAGAAT GCTGCGCTGA GTGCGTTGAG TAACGTCAGC ACCCACGTAG ACGGTAACAA	1260
	CCGCCACTTT GTAGATAAAG AAGATCGCGG CATGGCGAAA GAGATCGGCC AGTTTATGGA	1320
	TCAGTATCCG GAAATATTCG GTAAACCGGA ATACCAGAAA GATGGCTGGA GTTCGCCGAA	1380
	GACGGACGAC AAATCCTGGG CTAAAGCGCT GAGTAAACCG GATGATGACG GTATGACCGG	1440
	CGCCAGCATG GACAAATTCC GTCAGGCGAT GGGTATGATC AAAAGCGCGG TGGCGGGTGA	1500
30	TACCGGCAAT ACCAACCTGA ACCTGCGTGG CGCGGGCGGT GCATCGCTGG GTATCGATGC	1560
	GGCTGTCGTC GGCATAAAAA TAGCCAACAT GTCGCTGGGT AAGCTGGCCA ACGCCTGATA	1620

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ATCTGTGCTG GCCTGATAAA GCGGAAACGA AAAAAGAGAC GGGGAAGCCT GTCTCTTTTC 1680
 TTATTATGCG GTTTATGCGG TTACCTGGAC CGGTTAATCA TCGTCATCGA TCTGGTACAA 1740
 ACGCACATTT TCCCGTTCAT TCGCGTCGTT ACGCGCCACA ATCGCGATGG CATCTTCCTC 1800
 GTCGCTCAGA TTGCGCGGCT GATGGGGAAC GCCGGGTGGA ATATAGAGAA ACTCGCCGGC 1860
 5 CAGATGGAGA CACGTCTGCG ATAAATCTGT GCCGTAACGT GTTTCTATCC GCCCCTTTAG 1920
 CAGATAGATT GCGGTTTCGT AATCAACATG GTAATGCGGT TCCGCCTGTG CGCCGGCCGG 1980
 GATCACCACA ATATTCATAG AAAGCTGTCT TGCACCTACC GTATCGCGGG AGATACCGAC 2040
 AAAATAGGGC AGTTTTTGCG TGGTATCCGT GGGGTGTTCC GGCCTGACAA TCTTGAGTTG 2100
 GTTCGTCATC ATCTTTCTCC ATCTGGGCGA CCTGATCGGT T 2141

10

The hypersensitive response elicitor polypeptide or protein derived
 from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID.
 No. 3 as follows:

15

Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser
 1 5 10 15

Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln
 20 25 30

20

Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Asn
 35 40 45

Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met
 50 55 60

25

Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu
 65 70 75 80

Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu
 85 90 95

Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr
 100 105 110

30

Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro
 115 120 125

Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser
 130 135 140

35

Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln
 145 150 155 160

Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly
 165 170 175

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Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu
 180 185 190
 Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly
 195 200 205
 5 Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly
 210 215 220
 Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu
 225 230 235 240
 10 Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln
 245 250 255
 Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln
 260 265 270
 Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe
 275 280 285
 15 Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met
 290 295 300
 Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro
 305 310 315 320
 20 Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser
 325 330 335
 Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn
 340 345 350
 Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn
 355 360 365
 25 Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp
 370 375 380
 Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu
 385 390 395 400
 Gly Ala Ala

30

This hypersensitive response elicitor polypeptide or protein has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor polypeptide or protein has substantially no cysteine. The hypersensitive response elicitor polypeptide or protein derived from
 35 *Erwinia amylovora* is more fully described in Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*,"

Science 257:85-88 (1992), which is hereby incorporated by reference. The DNA molecule encoding this polypeptide or protein has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

5	AAGCTTCGGC ATGGCACGTT TGACCGTTGG GTCGGCAGGG TACGTTTGAA TTATTCATAA	60
	GAGGAATACG TTATGAGTCT GAATACAAGT GGGCTGGGAG CGTCAACGAT GCAAATTTCT	120
	ATCGGCGGTG CGGGCGGAAA TAACGGGTTG CTGGGTACCA GTCGCCAGAA TGCTGGGTTG	180
	GGTGGCAATT CTGCACTGGG GCTGGGCGGC GGTAATCAAA ATGATACCGT CAATCAGCTG	240
	GCTGGCTTAC TCACCGGCAT GATGATGATG ATGAGCATGA TGGGCGGTGG TGGGCTGATG	300
10	GGCGGTGGCT TAGGCGGTGG CTTAGGTAAT GGCTTGGGTG GCTCAGGTGG CCTGGGCGAA	360
	GGACTGTCTGA ACGCGCTGAA CGATATGTTA GGCGGTTCGC TGAACACGCT GGGCTCGAAA	420
	GGCGGCAACA ATACCACTTC AACAACAAAT TCCCCGCTGG ACCAGGCGCT GGGTATTAAC	480
	TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC	540
	CCGATGCAGC AGCTGCTGAA GATGTTGAGC GAGATAATGC AAAGCCTGTT TGGTGATGGG	600
15	CAAGATGGCA CCCAGGGCAG TTCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC	660
	GCCTATAAAA AAGGAGTCAC TGATGCGCTG TCGGGCCTGA TGGGTAATGG TCTGAGCCAG	720
	CTCCTTGGCA ACGGGGGACT GGGAGGTGGT CAGGGCGGTA ATGCTGGCAC GGGTCTTGAC	780
	GGTTCGTCGC TGGGCGGCAA AGGGCTGCAA AACCTGAGCG GGCCGGTGGA CTACCAGCAG	840
	TTAGGTAACG CCGTGGGTAC CGGTATCGGT ATGAAAGCGG GCATTGAGG GCTGAATGAT	900
20	ATCGGTACGC ACAGGCACAG TTCAACCCGT TCTTCGTCA ATAAAGGCGA TCGGGCGATG	960
	GCGAAGGAAA TCGGTCAGTT CATGGACCAG TATCTGAGG TGTTTGCCAA GCCGCAGTAC	1020
	CAGAAAGGCC CGGGTCAGGA GGTGAAAACC GATGACAAAT CATGGGCAAA AGCACTGAGC	1080
	AAGCCAGATG ACGACGGAAT GACACCAGCC AGTATGGAGC AGTTCAACAA AGCCAAGGGC	1140
	ATGATCAAAA GGCCCATGGC GGGTGATACC GGCAACGGCA ACCTGCAGGC ACGCGGTGCC	1200
25	GGTGGTTCTT CGCTGGGTAT TGATGCCATG ATGGCCGGTG ATGCCATTAA CAATATGGCA	1260
	CTTGGCAAGC TGGGCGCGGC TTAAGCTT	1288

Another potentially suitable hypersensitive response elicitor from *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,927, which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 5 as follows:

- 10 -

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        ATGTCAATTC TTACGCTTAA CAACAATACC TCGTCCTCGC CGGGTCTGTT CCAGTCCGGG      60
5      GGGGACAACG GGCTTGGTGG TCATAATGCA AATTCTGCGT TGGGGCAACA ACCCATCGAT      120
      CGGCAAACCA TTGAGCAAAT GGCTCAATTA TTGGCGGAAC TGTTAAAGTC ACTGCTATCG      180
      CCACAATCAG GTAATGCGGC AACCGGAGCC GGTGGCAATG ACCAGACTAC AGGAGTTGGT      240
10     AACGCTGGCG GCCTGAACGG ACGAAAAGGC ACAGCAGGAA CCACTCCGCA GTCTGACAGT      300
      CAGAACATGC TGAGTGAGAT GGGCAACAAC GGGCTGGATC AGGCCATCAC GCCCGATGGC      360
15     CAGGGCGGCG GGCAGATCGG CGATAATCCT TTAAGTAAAG CCATGCTGAA GCTTATTGCA      420
      CGCATGATGG ACGGCCAAAG CGATCAGTTT GGCCAACCTG GTACGGGCAA CAACAGTGCC      480
      TCTTCGGTA CTTCTTCATC TGGCGGTTCC CCTTTTAACG ATCTATCAGG GGGGAAGGCC      540
20     CCTTCGGCA ACTCCCCTTC CGGCAACTAC TCTCCGTC GTACCTTCTC ACCCCCATCC      600
      ACGCCAACGT CCCCTACCTC ACCGCTTGAT TTCCCTTCTT CTCCCACCAA AGCAGCCGGG      660
25     GGCAGCACGC CGGTAACCGA TCATCTGAC CCTGTTGGTA GCGCGGGCAT CGGGGCCGGA      720
      AATTCGGTGG CCTTCACCAG CGCCGGCGCT AATCAGACGG TGCTGCATGA CACCATTACC      780
      GTGAAAGCGG GTCAGGTGTT TGATGGCAAA GGACAAACCT TCACCGCCGG TTCAGAATTA      840
30     GGCATGGCG GCCAGTCTGA AAACCAGAAA CCGCTGTTTA TACTGGAAGA CGGTGCCAGC      900
      CTGAAAAACG TCACCATGGG CGACGACGGG GCGGATGGTA TTCATCTTTA CGGTGATGCC      960
35     AAAATAGACA ATCTGCACGT CACCAACGTG GGTGAGGACG CGATTACCGT TAAGCCAAAC      1020
      AGCGCGGGCA AAAAATCCCA CGTTGAAATC ACTAACAGTT CCTTCGAGCA CGCCTCTGAC      1080
      AAGATCCTGC AGCTGAATGC CGATACTAAC CTGAGCGTTG ACAACGTGAA GGCCAAAGAC      1140
40     TTTGGTACTT TTGTACGCAC TAACGGCGGT CAACAGGGTA ACTGGGATCT GAATCTGAGC      1200
      CATATCAGCG CAGAAGACGG TAAGTTCTCG TTCGTTAAAA GCGATAGCGA GGGGCTAAAC      1260
45     GTCAATACCA GTGATATCTC ACTGGGTGAT GTTGAAAACC ACTACAAAGT GCCGATGTCC      1320
      GCCAACCTGA AGGTGGCTGA ATGA                                     1344

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See GenBank Accession No. U94513. The isolated DNA molecule of the present
 invention encodes a hypersensitive response elicitor protein or polypeptide having an
 amino acid sequence of SEQ. ID. No. 6 as follows:

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Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu
1           5           10           15
55
Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser
20           25           30
60
Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala
35           40           45

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	Gln	Leu	Leu	Ala	Glu	Leu	Leu	Lys	Ser	Leu	Leu	Ser	Pro	Gln	Ser	Gly	
	50						55					60					
5	Asn	Ala	Ala	Thr	Gly	Ala	Gly	Gly	Asn	Asp	Gln	Thr	Thr	Gly	Val	Gly	
	65					70					75					80	
	Asn	Ala	Gly	Gly	Leu	Asn	Gly	Arg	Lys	Gly	Thr	Ala	Gly	Thr	Thr	Pro	
					85					90					95		
10	Gln	Ser	Asp	Ser	Gln	Asn	Met	Leu	Ser	Glu	Met	Gly	Asn	Asn	Gly	Leu	
				100					105					110			
	Asp	Gln	Ala	Ile	Thr	Pro	Asp	Gly	Gln	Gly	Gly	Gly	Gln	Ile	Gly	Asp	
15			115					120					125				
	Asn	Pro	Leu	Leu	Lys	Ala	Met	Leu	Lys	Leu	Ile	Ala	Arg	Met	Met	Asp	
		130					135					140					
20	Gly	Gln	Ser	Asp	Gln	Phe	Gly	Gln	Pro	Gly	Thr	Gly	Asn	Asn	Ser	Ala	
	145					150					155					160	
	Ser	Ser	Gly	Thr	Ser	Ser	Ser	Gly	Gly	Ser	Pro	Phe	Asn	Asp	Leu	Ser	
					165					170					175		
25	Gly	Gly	Lys	Ala	Pro	Ser	Gly	Asn	Ser	Pro	Ser	Gly	Asn	Tyr	Ser	Pro	
				180					185					190			
	Val	Ser	Thr	Phe	Ser	Pro	Pro	Ser	Thr	Pro	Thr	Ser	Pro	Thr	Ser	Pro	
30			195					200					205				
	Leu	Asp	Phe	Pro	Ser	Ser	Pro	Thr	Lys	Ala	Ala	Gly	Gly	Ser	Thr	Pro	
		210					215					220					
35	Val	Thr	Asp	His	Pro	Asp	Pro	Val	Gly	Ser	Ala	Gly	Ile	Gly	Ala	Gly	
	225					230					235					240	
	Asn	Ser	Val	Ala	Phe	Thr	Ser	Ala	Gly	Ala	Asn	Gln	Thr	Val	Leu	His	
				245					250					255			
40	Asp	Thr	Ile	Thr	Val	Lys	Ala	Gly	Gln	Val	Phe	Asp	Gly	Lys	Gly	Gln	
				260					265					270			
	Thr	Phe	Thr	Ala	Gly	Ser	Glu	Leu	Gly	Asp	Gly	Gly	Gln	Ser	Glu	Asn	
45			275					280					285				
	Gln	Lys	Pro	Leu	Phe	Ile	Leu	Glu	Asp	Gly	Ala	Ser	Leu	Lys	Asn	Val	
		290					295					300					
50	Thr	Met	Gly	Asp	Asp	Gly	Ala	Asp	Gly	Ile	His	Leu	Tyr	Gly	Asp	Ala	
	305					310					315					320	
	Lys	Ile	Asp	Asn	Leu	His	Val	Thr	Asn	Val	Gly	Glu	Asp	Ala	Ile	Thr	
				325					330					335			
55	Val	Lys	Pro	Asn	Ser	Ala	Gly	Lys	Lys	Ser	His	Val	Glu	Ile	Thr	Asn	
				340					345					350			

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Ser Ser Phe Glu His Ala Ser Asp Lys Ile Leu Gln Leu Asn Ala Asp
 355 360 365
 5 Thr Asn Leu Ser Val Asp Asn Val Lys Ala Lys Asp Phe Gly Thr Phe
 370 375 380
 Val Arg Thr Asn Gly Gly Gln Gln Gly Asn Trp Asp Leu Asn Leu Ser
 385 390 395 400
 10 His Ile Ser Ala Glu Asp Gly Lys Phe Ser Phe Val Lys Ser Asp Ser
 405 410 415
 Glu Gly Leu Asn Val Asn Thr Ser Asp Ile Ser Leu Gly Asp Val Glu
 420 425 430
 15 Asn His Tyr Lys Val Pro Met Ser Ala Asn Leu Lys Val Ala Glu
 435 440 445

20 This protein or polypeptide is acidic, rich in glycine and serine, and lacks cysteine. It is also heat stable, protease sensitive, and suppressed by inhibitors of plant metabolism. The protein or polypeptide of the present invention has a predicted molecular size of ca. 4.5 kDa.

Another potentially suitable hypersensitive response elicitor from
 25 *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,663, which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 7 as follows:

30 ATGGAATTAA AATCACTGGG AACTGAACAC AAGGCGGCAG TACACACAGC GGCGCACAAAC 60
 CCTGTGGGGC ATGGTGTGTC CTTACAGCAG GGCAGCAGCA GCAGCAGCCC GCAAAATGCC 120
 GCTGCATCAT TGGCGGCAGA AGGCAAAAAT CGTGGGAAAA TGCCGAGAAT TCACCAGCCA 180
 35 TCTACTGCGG CTGATGGTAT CAGCGCTGCT CACCAGCAAA AGAAATCCTT CAGTCTCAGG 240
 GGCTGTTTGG GGACGAAAAA ATTTTCCAGA TCGGCACCGC AGGGCCAGCC AGGTACCACC 300
 CACAGCAAAG GGGCAACATT GCGCGATCTG CTGGCGCGGG ACGACGGCGA AACGCAGCAT 360
 40 GAGGCGGCCG CGCCAGATGC GGC GCGTTTG ACCCGTTCGG GCGGCGTCAA ACGCCGCAAT 420
 ATGGACGACA TGGCCGGGCG GCCAATGGTG AAAGGTGGCA GCGGCGAAGA TAAGGTACCA 480
 45 ACGCAGCAAA AACGGCATCA GCTGAACAAT TTTGGCCAGA TGCGCCAAAC GATGTTGAGC 540
 AAAATGGCTC ACCCGGCTTC AGCCAACGCC GGC GATCGCC TGCAGCATT CACCGCCGAC 600
 ATCCCGGGTA GCCACCACGA AATCAAGGAA GAACCGGTTG GCTCCACCAG CAAGGCAACA 660
 50 ACGGCCCCAG CAGACAGAGT GGAAATCGCT CAGGAAGATG ACGACAGCGA ATTCCAGCAA 720
 CTGCATCAAC AGCGGCTGGC GCGCGAACGG GAAAATCCAC CGCAGCCGCC CAAACTCGGC 780
 55 GTTGCCACAC CGATTAGCGC CAGGTTTCAG CCCAACTGA CTGCGGTTGC GGAAAGCGTC 840

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	CTTGAGGGGA CAGATACCAC GCAGTCACCC CTTAAGCCGC AATCAATGCT GAAAGGAAGT	900
5	GGAGCCGGGG TAACGCCGCT GGCGGTAACG CTGGATAAAG GCAAGTTGCA GCTGGCACCG	960
	GATAATCCAC CCGCGCTCAA TACGTTGTTG AAGCAGACAT TGGGTAAAGA CACCCAGCAC	1020
	TATCTGGCGC ACCATGCCAG CAGCGACGGT AGCCAGCATC TGCTGCTGGA CAACAAAGGC	1080
10	CACCTGTTTG ATATCAAAAG CACCGCCACC AGCTATAGCG TGCTGCACAA CAGCCACCCC	1140
	GGTGAGATAA AGGGCAAGCT GGCGCAGGCG GGTACTGGCT CCGTCAGCGT AGACGGTAAA	1200
15	AGCGGCAAGA TCTCGCTGGG GAGCGGTACG CAAAGTCACA ACAAACAAT GCTAAGCCAA	1260
	CCGGGGGAAG CGCACCCTTC CTTATTAACC GGCATTGGC AGCATCCTGC TGGCGCAGCG	1320
	CGGCCGCAGG GCGAGTCAAT CCGCCTGCAT GACGACAAAA TTCATATCCT GCATCCGGAG	1380
20	CTGGGCGTAT GGCAATCTGC GGATAAAGAT ACCCAGAGCC AGCTGTCTCG CCAGGCAGAC	1440
	GGTAAGCTCT ATGCGCTGAA AGACAACCGT ACCCTGCAAA ACCTCTCCGA TAATAAATCC	1500
	TCAGAAAAGC TGGTCGATAA AATCAAATCG TATTCCGTTG ATCAGCGGGG GCAGGTGGCG	1560
25	ATCCTGACGG ATACTCCCGG CCGCCATAAG ATGAGTATTA TGCCCTCGCT GGATGCTTCC	1620
	CCGGAGAGCC ATATTTCCCT CAGCCTGCAT TTTGCCGATG CCCACCAGGG GTTATTGCAC	1680
30	GGGAAGTCGG AGCTTGAGGC ACAATCTGTC GCGATCAGCC ATGGGCGACT GGTGTGGCC	1740
	GATAGCGAAG GCAAGCTGTT TAGCGCCGCC ATTCCGAAGC AAGGGGATGG AAACGAACTG	1800
	AAAATGAAAG CCATGCCTCA GCATGCGCTC GATGAACATT TTGGTCATGA CCACCAGATT	1860
35	TCTGGATTTT TCCATGACGA CCACGGCCAG CTTAATGCGC TGGTGAAAAA TAACCTCAGG	1920
	CAGCAGCATG CCTGCCCCTT GGGTAACGAT CATCAGTTTC ACCCCGGCTG GAACCTGACT	1980
40	GATGCGCTGG TTATCGACAA TCAGCTGGGG CTGCATCATA CCAATCCTGA ACCGCATGAG	2040
	ATTCTTGATA TGGGGCATT AGGCAGCCTG GCGTTACAGG AGGGCAAGCT TCACTATTTT	2100
45	GACCAGCTGA CCAAAGGGTG GACTGGCGCG GAGTCAGATT GTAAGCAGCT GAAAAAGGC	2160
	CTGGATGGAG CAGCTTATCT ACTGAAAGAC GGTGAAGTGA AACGCCTGAA TATTAATCAG	2220
	AGCACCTCCT CTATCAAGCA CGGAACGGAA AACGTTTTTT CGCTGCCGCA TGTGCGCAAT	2280
50	AAACCGGAGC CGGGAGATGC CCTGCAAGGG CTGAATAAAG ACGATAAGGC CCAGGCCATG	2340
	GCGGTGATTG GGGTAAATAA ATACCTGGCG CTGACGGAAA AAGGGGACAT TCGCTCCTTC	2400
55	CAGATAAAAC CCGGCACCCA GCAGTTGGAG CGGCCGGCAC AAATCTCTAG CCGCGAAGGT	2460
	ATCAGCGGCG AACTGAAAGA CATTTCATGTC GACCACAAGC AGAACCTGTA TGCCTTGACC	2520
	CACGAGGGAG AGGTGTTTCA TCAGCCCGCT GAAGCCTGGC AGAATGGTGC CGAAAGCAGC	2580
60	AGCTGGCACA AACTGGCGTT GCCACAGAGT GAAAGTAAGC TAAAAAGTCT GGACATGAGC	2640
	CATGAGCACA AACCGATTGC CACCTTTGAA GACGGTAGCC AGCATCAGCT GAAGGCTGGC	2700
65	GGCTGGCACG CCTATGCGGC ACCTGAACGC GGGCCGCTGG CGGTGGGTAC CAGCGGTTCA	2760

	CAAACCGTCT TTAACCGACT AATGCAGGGG GTGAAAGGCA AGGTGATCCC AGGCAGCGGG	2820
	TTGACGGTTA AGCTCTCGGC TCAGACGGGG GGAATGACCG GCGCCGAAGG GCGCAAGGTC	2880
5	AGCAGTAAAT TTTCCGAAAG GATCCGCGCC TATGCGTTCA ACCCAACAAT GTCCACGCCG	2940
	CGACCGATTA AAAATGCTGC TTATGCCACA CAGCACGGCT GGCAGGGGCG TGAGGGGTTG	3000
10	AAGCCGTTGT ACGAGATGCA GGGAGCGCTG ATTAAACAAC TGGATGCGCA TAACGTTCTG	3060
	CATAACGCGC CACAGCCAGA TTTGCAGAGC AAATGGAAA CTCTGGATTT AGGCGAACAT	3120
	GGCGCAGAAT TGCTTAACGA CATGAAGCGC TTCCGCGACG AACTGGAGCA GAGTGCAACC	3180
15	CGTTCGGTGA CCGTTTTAGG TCAACATCAG GGAGTGCTAA AAAGCAACGG TGAAATCAAT	3240
	AGCGAATTTA AGCCATCGCC CGGCAAGGCG TTGGTCCAGA GCTTTAACGT CAATCGCTCT	3300
20	GGTCAGGATC TAAGCAAGTC ACTGCAACAG GCAGTACATG CCACGCCGCC ATCCGCAGAG	3360
	AGTAACTGC AATCCATGCT GGGGCACTTT GTCAGTGCCG GGGTGGATAT GAGTCATCAG	3420
	AAGGGCGAGA TCCCGCTGGG CCGCCAGCGC GATCCGAATG ATAAAACCGC ACTGACCAAA	3480
25	TCGCGTTTAA TTTAGATAC CGTGACCATC GGTGAACTGC ATGAACTGGC CGATAAGGCG	3540
	AAACTGGTAT CTGACCATAA ACCCGATGCC GATCAGATAA AACAGCTGCG CCAGCAGTTC	3600
30	GATACGCTGC GTGAAAAGCG GTATGAGAGC AATCCGGTGA AGCATTACAC CGATATGGGC	3660
	TTCACCCATA ATAAGGCGCT GGAAGCAAAC TATGATGCGG TCAAAGCCTT TATCAATGCC	3720
	TTTAAGAAAG AGCACCACGG CGTCAATCTG ACCACGCGTA CCGTACTGGA ATCACAGGGC	3780
35	AGTGCAGGAGC TGGCGAAGAA GCTCAAGAAT ACGCTGTTGT CCCTGGACAG TGGTGAAAGT	3840
	ATGAGCTTCA GCCGGTCATA TGGCGGGGGC GTCAGCACTG TCTTTGTGCC TACCCCTAGC	3900
40	AAGAAGGTGC CAGTTCCGGT GATCCCCGGA GCCGGCATCA CGCTGGATCG CGCCTATAAC	3960
	CTGAGCTTCA GTCGTACCAG CGGCGGATTG AACGTCAGTT TTGGCCGCGA CGGCGGGGTG	4020
	AGTGGTAACA TCATGGTCGC TACCGGCCAT GATGTGATGC CCTATATGAC CGGTAAGAAA	4080
45	ACCACTGCAG GTAACGCCAG TGACTGGTTG AGCGCAAAAC ATAAAATCAG CCCGGACTTG	4140
	CGTATCGGCG CTGCTGTGAG TGGCACCTG CAAGGAACGC TACAAAACAG CCTGAAGTTT	4200
50	AAGCTGACAG AGGATGAGCT GCCTGGCTTT ATCCATGGCT TGACGCATGG CACGTTGACC	4260
	CCGGCAGAAC TGTTGCAAAA GGGGATCGAA CATCAGATGA AGCAGGGCAG CAAACTGACG	4320
	TTTAGCGTCG ATACCTCGGC AAATCTGGAT CTGCGTGCCG GTATCAATCT GAACGAAGAC	4380
55	GGCAGTAAAC CAAATGGTGT CACTGCCCGT GTTTCTGCCG GGCTAAGTGC ATCGGCAAAC	4440
	CTGGCCGCCG GCTCGCGTGA ACGCAGCACC ACCTCTGGCC AGTTTGGCAG CACGACTTCG	4500
60	GCCAGCAATA ACCGCCAAC CTCCTCAAC GGGGTCGGCG CGGGTGCTAA CCTGACGGCT	4560
	GCTTTAGGGG TTGCCCATTC ATCTACGCAT GAAGGGAAC CGGTCCGGAT CTTCCCGGCA	4620
	TTTACCTCGA CCAATGTTTC GGCAGCGCTG GCGCTGGATA ACCGTACCTC ACAGAGTATC	4680
65	AGCCTGGAAT TGAAGCGCGC GGAGCCGGTG ACCAGCAACG ATATCAGCGA GTTGACCTCC	4740

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ACGCTGGGAA AACACTTTAA GGATAGCGCC ACAACGAAGA TGCTTGCCGC TCTCAAAGAG 4800
 TTAGATGACG CTAAGCCCGC TGAACAACTG CATATTTTAC AGCAGCATT T CAGTGCAAAA 4860
 5 GATGTCGTGC GTGATGAACG CTACGAGGCG GTGCGCAACC TGAAAAAACT GGTGATACGT 4920
 CAACAGGCTG CGGACAGCCA CAGCATGGAA TTAGGATCTG CCAGTCACAG CACGACCTAC 4980
 10 AATAATCTGT CGAGAATAAA TAATGACGGC ATTGTGAGC TGCTACACAA ACATTTCGAT 5040
 GCGGCATTAC CAGCAAGCAG TGCCAAACGT CTTGGTGAAA TGATGAATAA CGATCCGGCA 5100
 CTGAAAGATA TTATTAAGCA GCTGCAAAGT ACGCCGTTCA GCAGCGCCAG CGTGTCGATG 5160
 15 GAGCTGAAAG ATGGTCTGCG TGAGCAGACG GAAAAAGCAA TACTGGACGG TAAGGTCGGT 5220
 CGTGAAGAAG TGGGAGTACT TTTCCAGGAT CGTAACAACT TCGTGTTAA ATCGGTCAGC 5280
 20 GTCAGTCAGT CCGTCAGCAA AAGCGAAGGC TTCAATACCC CAGCGCTGTT ACTGGGGACG 5340
 AGCAACAGCG CTGCTATGAG CATGGAGCGC AACATCGGAA CCATTAATTT TAAATACGGC 5400
 CAGGATCAGA ACACCCACG GCGATTTACC CTGGAGGGTG GAATAGCTCA GGCTAATCCG 5460
 25 CAGGTCGCAT CTGCGCTTAC TGATTGAAG AAGGAAGGGC TGGAAATGAA GAGCTAA 5517

This DNA molecule is known as the dspE gene for *Erwinia amylovora*. This isolated
 30 DNA molecule of the present invention encodes a protein or polypeptide which elicits
 a plant pathogen's hypersensitive response having an amino acid sequence of SEQ.
 ID. No. 8 as follows:

35 Met Glu Leu Lys Ser Leu Gly Thr Glu His Lys Ala Ala Val His Thr
 1 5 10 15
 Ala Ala His Asn Pro Val Gly His Gly Val Ala Leu Gln Gln Gly Ser
 20 25 30
 40 Ser Ser Ser Ser Pro Gln Asn Ala Ala Ala Ser Leu Ala Ala Glu Gly
 35 40 45
 Lys Asn Arg Gly Lys Met Pro Arg Ile His Gln Pro Ser Thr Ala Ala
 50 55 60
 45 Asp Gly Ile Ser Ala Ala His Gln Gln Lys Lys Ser Phe Ser Leu Arg
 65 70 75 80
 Gly Cys Leu Gly Thr Lys Lys Phe Ser Arg Ser Ala Pro Gln Gly Gln
 50 85 90 95
 Pro Gly Thr Thr His Ser Lys Gly Ala Thr Leu Arg Asp Leu Leu Ala
 100 105 110
 55 Arg Asp Asp Gly Glu Thr Gln His Glu Ala Ala Ala Pro Asp Ala Ala
 115 120 125
 Arg Leu Thr Arg Ser Gly Gly Val Lys Arg Arg Asn Met Asp Asp Met
 130 135 140
 60

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Ala Gly Arg Pro Met Val Lys Gly Gly Ser Gly Glu Asp Lys Val Pro
 145 150 155 160
 5 Thr Gln Gln Lys Arg His Gln Leu Asn Asn Phe Gly Gln Met Arg Gln
 165 170 175
 Thr Met Leu Ser Lys Met Ala His Pro Ala Ser Ala Asn Ala Gly Asp
 180 185 190
 10 Arg Leu Gln His Ser Pro Pro His Ile Pro Gly Ser His His Glu Ile
 195 200 205
 Lys Glu Glu Pro Val Gly Ser Thr Ser Lys Ala Thr Thr Ala His Ala
 210 215 220
 15 Asp Arg Val Glu Ile Ala Gln Glu Asp Asp Asp Ser Glu Phe Gln Gln
 225 230 235 240
 Leu His Gln Gln Arg Leu Ala Arg Glu Arg Glu Asn Pro Pro Gln Pro
 245 250 255
 20 Pro Lys Leu Gly Val Ala Thr Pro Ile Ser Ala Arg Phe Gln Pro Lys
 260 265 270
 25 Leu Thr Ala Val Ala Glu Ser Val Leu Glu Gly Thr Asp Thr Thr Gln
 275 280 285
 Ser Pro Leu Lys Pro Gln Ser Met Leu Lys Gly Ser Gly Ala Gly Val
 290 295 300
 30 Thr Pro Leu Ala Val Thr Leu Asp Lys Gly Lys Leu Gln Leu Ala Pro
 305 310 315 320
 35 Asp Asn Pro Pro Ala Leu Asn Thr Leu Leu Lys Gln Thr Leu Gly Lys
 325 330 335
 Asp Thr Gln His Tyr Leu Ala His His Ala Ser Ser Asp Gly Ser Gln
 340 345 350
 40 His Leu Leu Leu Asp Asn Lys Gly His Leu Phe Asp Ile Lys Ser Thr
 355 360 365
 Ala Thr Ser Tyr Ser Val Leu His Asn Ser His Pro Gly Glu Ile Lys
 370 375 380
 45 Gly Lys Leu Ala Gln Ala Gly Thr Gly Ser Val Ser Val Asp Gly Lys
 385 390 395 400
 50 Ser Gly Lys Ile Ser Leu Gly Ser Gly Thr Gln Ser His Asn Lys Thr
 405 410 415
 Met Leu Ser Gln Pro Gly Glu Ala His Arg Ser Leu Leu Thr Gly Ile
 420 425 430
 55 Trp Gln His Pro Ala Gly Ala Ala Arg Pro Gln Gly Glu Ser Ile Arg
 435 440 445
 Leu His Asp Asp Lys Ile His Ile Leu His Pro Glu Leu Gly Val Trp
 450 455 460
 60 Gln Ser Ala Asp Lys Asp Thr His Ser Gln Leu Ser Arg Gln Ala Asp
 465 470 475 480
 65 Gly Lys Leu Tyr Ala Leu Lys Asp Asn Arg Thr Leu Gln Asn Leu Ser
 485 490 495

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	Asp	Asn	Lys	Ser	Ser	Glu	Lys	Leu	Val	Asp	Lys	Ile	Lys	Ser	Tyr	Ser	
				500					505					510			
5	Val	Asp	Gln	Arg	Gly	Gln	Val	Ala	Ile	Leu	Thr	Asp	Thr	Pro	Gly	Arg	
			515					520					525				
	His	Lys	Met	Ser	Ile	Met	Pro	Ser	Leu	Asp	Ala	Ser	Pro	Glu	Ser	His	
			530				535						540				
10	Ile	Ser	Leu	Ser	Leu	His	Phe	Ala	Asp	Ala	His	Gln	Gly	Leu	Leu	His	
	545					550					555					560	
	Gly	Lys	Ser	Glu	Leu	Glu	Ala	Gln	Ser	Val	Ala	Ile	Ser	His	Gly	Arg	
					565					570					575		
15	Leu	Val	Val	Ala	Asp	Ser	Glu	Gly	Lys	Leu	Phe	Ser	Ala	Ala	Ile	Pro	
				580					585						590		
	Lys	Gln	Gly	Asp	Gly	Asn	Glu	Leu	Lys	Met	Lys	Ala	Met	Pro	Gln	His	
20			595					600					605				
	Ala	Leu	Asp	Glu	His	Phe	Gly	His	Asp	His	Gln	Ile	Ser	Gly	Phe	Phe	
		610					615					620					
25	His	Asp	Asp	His	Gly	Gln	Leu	Asn	Ala	Leu	Val	Lys	Asn	Asn	Phe	Arg	
	625					630					635					640	
	Gln	Gln	His	Ala	Cys	Pro	Leu	Gly	Asn	Asp	His	Gln	Phe	His	Pro	Gly	
					645					650					655		
30	Trp	Asn	Leu	Thr	Asp	Ala	Leu	Val	Ile	Asp	Asn	Gln	Leu	Gly	Leu	His	
				660					665					670			
	His	Thr	Asn	Pro	Glu	Pro	His	Glu	Ile	Leu	Asp	Met	Gly	His	Leu	Gly	
35			675					680					685				
	Ser	Leu	Ala	Leu	Gln	Glu	Gly	Lys	Leu	His	Tyr	Phe	Asp	Gln	Leu	Thr	
		690					695					700					
40	Lys	Gly	Trp	Thr	Gly	Ala	Glu	Ser	Asp	Cys	Lys	Gln	Leu	Lys	Lys	Gly	
	705					710					715					720	
	Leu	Asp	Gly	Ala	Ala	Tyr	Leu	Leu	Lys	Asp	Gly	Glu	Val	Lys	Arg	Leu	
					725					730					735		
45	Asn	Ile	Asn	Gln	Ser	Thr	Ser	Ser	Ile	Lys	His	Gly	Thr	Glu	Asn	Val	
				740					745					750			
	Phe	Ser	Leu	Pro	His	Val	Arg	Asn	Lys	Pro	Glu	Pro	Gly	Asp	Ala	Leu	
50			755					760					765				
	Gln	Gly	Leu	Asn	Lys	Asp	Asp	Lys	Ala	Gln	Ala	Met	Ala	Val	Ile	Gly	
		770					775					780					
55	Val	Asn	Lys	Tyr	Leu	Ala	Leu	Thr	Glu	Lys	Gly	Asp	Ile	Arg	Ser	Phe	
	785					790					795					800	
	Gln	Ile	Lys	Pro	Gly	Thr	Gln	Gln	Leu	Glu	Arg	Pro	Ala	Gln	Thr	Leu	
					805					810					815		
60	Ser	Arg	Glu	Gly	Ile	Ser	Gly	Glu	Leu	Lys	Asp	Ile	His	Val	Asp	His	
				820					825					830			
	Lys	Gln	Asn	Leu	Tyr	Ala	Leu	Thr	His	Glu	Gly	Glu	Val	Phe	His	Gln	
65			835					840					845				

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Pro Arg Glu Ala Trp Gln Asn Gly Ala Glu Ser Ser Ser Trp His Lys
 850 855 860

5 Leu Ala Leu Pro Gln Ser Glu Ser Lys Leu Lys Ser Leu Asp Met Ser
 865 870 875 880

His Glu His Lys Pro Ile Ala Thr Phe Glu Asp Gly Ser Gln His Gln
 885 890 895

10 Leu Lys Ala Gly Gly Trp His Ala Tyr Ala Ala Pro Glu Arg Gly Pro
 900 905 910

15 Leu Ala Val Gly Thr Ser Gly Ser Gln Thr Val Phe Asn Arg Leu Met
 915 920 925

Gln Gly Val Lys Gly Lys Val Ile Pro Gly Ser Gly Leu Thr Val Lys
 930 935 940

20 Leu Ser Ala Gln Thr Gly Gly Met Thr Gly Ala Glu Gly Arg Lys Val
 945 950 955 960

Ser Ser Lys Phe Ser Glu Arg Ile Arg Ala Tyr Ala Phe Asn Pro Thr
 965 970 975

25 Met Ser Thr Pro Arg Pro Ile Lys Asn Ala Ala Tyr Ala Thr Gln His
 980 985 990

Gly Trp Gln Gly Arg Glu Gly Leu Lys Pro Leu Tyr Glu Met Gln Gly
 995 1000 1005

30 Ala Leu Ile Lys Gln Leu Asp Ala His Asn Val Arg His Asn Ala Pro
 1010 1015 1020

35 Gln Pro Asp Leu Gln Ser Lys Leu Glu Thr Leu Asp Leu Gly Glu His
 1025 1030 1035 1040

Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu
 1045 1050 1055

40 Gln Ser Ala Thr Arg Ser Val Thr Val Leu Gly Gln His Gln Gly Val
 1060 1065 1070

Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly
 1075 1080 1085

45 Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu
 1090 1095 1100

50 Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu
 1105 1110 1115 1120

Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp
 1125 1130 1135

55 Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro
 1140 1145 1150

60 Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val
 1155 1160 1165

Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser
 1170 1175 1180

65 Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe
 1185 1190 1195 1200

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	Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr	
	1205	1210 1215
5	Thr Asp Met Gly Phe Thr His Asn Lys Ala Leu Glu Ala Asn Tyr Asp	
	1220	1225 1230
	Ala Val Lys Ala Phe Ile Asn Ala Phe Lys Lys Glu His His Gly Val	
	1235	1240 1245
10	Asn Leu Thr Thr Arg Thr Val Leu Glu Ser Gln Gly Ser Ala Glu Leu	
	1250	1255 1260
	Ala Lys Lys Leu Lys Asn Thr Leu Leu Ser Leu Asp Ser Gly Glu Ser	
15	1265	1270 1275 1280
	Met Ser Phe Ser Arg Ser Tyr Gly Gly Gly Val Ser Thr Val Phe Val	
	1285	1290 1295
20	Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly	
	1300	1305 1310
	Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly	
	1315	1320 1325
25	Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile	
	1330	1335 1340
	Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys	
30	1345	1350 1355 1360
	Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile	
	1365	1370 1375
35	Ser Pro Asp Leu Arg Ile Gly Ala Ala Val Ser Gly Thr Leu Gln Gly	
	1380	1385 1390
	Thr Leu Gln Asn Ser Leu Lys Phe Lys Leu Thr Glu Asp Glu Leu Pro	
	1395	1400 1405
40	Gly Phe Ile His Gly Leu Thr His Gly Thr Leu Thr Pro Ala Glu Leu	
	1410	1415 1420
	Leu Gln Lys Gly Ile Glu His Gln Met Lys Gln Gly Ser Lys Leu Thr	
45	1425	1430 1435 1440
	Phe Ser Val Asp Thr Ser Ala Asn Leu Asp Leu Arg Ala Gly Ile Asn	
	1445	1450 1455
50	Leu Asn Glu Asp Gly Ser Lys Pro Asn Gly Val Thr Ala Arg Val Ser	
	1460	1465 1470
	Ala Gly Leu Ser Ala Ser Ala Asn Leu Ala Ala Gly Ser Arg Glu Arg	
	1475	1480 1485
55	Ser Thr Thr Ser Gly Gln Phe Gly Ser Thr Thr Ser Ala Ser Asn Asn	
	1490	1495 1500
	Arg Pro Thr Phe Leu Asn Gly Val Gly Ala Gly Ala Asn Leu Thr Ala	
60	1505	1510 1515 1520
	Ala Leu Gly Val Ala His Ser Ser Thr His Glu Gly Lys Pro Val Gly	
	1525	1530 1535

- 20 -

Ile Phe Pro Ala Phe Thr Ser Thr Asn Val Ser Ala Ala Leu Ala Leu
 1540 1545 1550
 5 Asp Asn Arg Thr Ser Gln Ser Ile Ser Leu Glu Leu Lys Arg Ala Glu
 1555 1560 1565
 Pro Val Thr Ser Asn Asp Ile Ser Glu Leu Thr Ser Thr Leu Gly Lys
 1570 1575 1580
 10 His Phe Lys Asp Ser Ala Thr Thr Lys Met Leu Ala Ala Leu Lys Glu
 1585 1590 1595 1600
 Leu Asp Asp Ala Lys Pro Ala Glu Gln Leu His Ile Leu Gln Gln His
 1605 1610 1615
 15 Phe Ser Ala Lys Asp Val Val Gly Asp Glu Arg Tyr Glu Ala Val Arg
 1620 1625 1630
 Asn Leu Lys Lys Leu Val Ile Arg Gln Gln Ala Ala Asp Ser His Ser
 1635 1640 1645
 20 Met Glu Leu Gly Ser Ala Ser His Ser Thr Thr Tyr Asn Asn Leu Ser
 1650 1655 1660
 Arg Ile Asn Asn Asp Gly Ile Val Glu Leu Leu His Lys His Phe Asp
 1665 1670 1675 1680
 Ala Ala Leu Pro Ala Ser Ser Ala Lys Arg Leu Gly Glu Met Met Asn
 1685 1690 1695
 30 Asn Asp Pro Ala Leu Lys Asp Ile Ile Lys Gln Leu Gln Ser Thr Pro
 1700 1705 1710
 Phe Ser Ser Ala Ser Val Ser Met Glu Leu Lys Asp Gly Leu Arg Glu
 1715 1720 1725
 35 Gln Thr Glu Lys Ala Ile Leu Asp Gly Lys Val Gly Arg Glu Glu Val
 1730 1735 1740
 Gly Val Leu Phe Gln Asp Arg Asn Asn Leu Arg Val Lys Ser Val Ser
 1745 1750 1755 1760
 Val Ser Gln Ser Val Ser Lys Ser Glu Gly Phe Asn Thr Pro Ala Leu
 1765 1770 1775
 45 Leu Leu Gly Thr Ser Asn Ser Ala Ala Met Ser Met Glu Arg Asn Ile
 1780 1785 1790
 Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pro Arg Arg
 1795 1800 1805
 50 Phe Thr Leu Glu Gly Gly Ile Ala Gln Ala Asn Pro Gln Val Ala Ser
 1810 1815 1820
 Ala Leu Thr Asp Leu Lys Lys Glu Gly Leu Glu Met Lys Ser
 1825 1830 1835

This protein or polypeptide is about 198 kDa and has a pI of 8.98.

60 The present invention relates to an isolated DNA molecule having a
 nucleotide sequence of SEQ. ID. No. 9 as follows:

- 21 -

5 ATGACATCGT CACAGCAGCG GGTGAAAGG TTTTACAGT ATTTCTCCGC CGGGTGTAAG 60
 ACGCCCATAC ATCTGAAAGA CGGGGTGTGC GCCCTGTATA ACGAACAAGA TGAGGAGGCG 120
 GCGGTGCTGG AAGTACCGCA ACACAGCGAC AGCCTGTTAC TACACTGCCG AATCATTGAG 180
 GCTGACCCAC AAACCTCAAT AACCTGTAT TCGATGCTAT TACAGCTGAA TTTTGAAATG 240
 10 GCGGCCATGC GCGGCTGTTG GCTGGCGCTG GATGAACTGC ACAACGTGCG TTTATGTTTT 300
 CAGCAGTCGC TGGAGCATCT GGATGAAGCA AGTTTTAGCG ATATCGTTAG CGGCTTCATC 360
 GAACATGCGG CAGAAGTGCG TGAGTATATA GCGCAATTAG ACGAGAGTAG CGCGGCATAA 420
 15

This is known as the dspF gene. This isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 10 as follows:

20 Met Thr Ser Ser Gln Gln Arg Val Glu Arg Phe Leu Gln Tyr Phe Ser
 1 5 10 15
 25 Ala Gly Cys Lys Thr Pro Ile His Leu Lys Asp Gly Val Cys Ala Leu
 20 25 30
 Tyr Asn Glu Gln Asp Glu Glu Ala Ala Val Leu Glu Val Pro Gln His
 35 40 45
 30 Ser Asp Ser Leu Leu Leu His Cys Arg Ile Ile Glu Ala Asp Pro Gln
 50 55 60
 Thr Ser Ile Thr Leu Tyr Ser Met Leu Leu Gln Leu Asn Phe Glu Met
 65 70 75 80
 35 Ala Ala Met Arg Gly Cys Trp Leu Ala Leu Asp Glu Leu His Asn Val
 85 90 95
 40 Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe
 100 105 110
 Ser Asp Ile Val Ser Gly Phe Ile Glu His Ala Ala Glu Val Arg Glu
 115 120 125
 45 Tyr Ile Ala Gln Leu Asp Glu Ser Ser Ala Ala
 130 135

This protein or polypeptide is about 16 kDa and has a pI of 4.45.

50 The hypersensitive response elicitor polypeptide or protein derived
 from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID.
 No. 11 as follows:

55 Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met
 1 5 10 15

- 22 -

Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser
20 25 30

5 Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
35 40 45

Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
50 55 60

Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val
65 70 75 80

10 Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
85 90 95

Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
100 105 110

15 Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
115 120 125

Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met
130 135 140

Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
145 150 155 160

20 Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
165 170 175

Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
180 185 190

25 Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly
195 200 205

Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
210 215 220

Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser
225 230 235 240

30 Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp
245 250 255

Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val
260 265 270

35 Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
275 280 285

Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala
290 295 300

Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
305 310 315 320

- 23 -

Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg
 325 330 335

Asn Gln Ala Ala Ala
 340

5

This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine.

Further information about the hypersensitive response elicitor derived from

Pseudomonas syringae is found in He, S. Y., H. C. Huang, and A. Collmer,

10 "*Pseudomonas syringae* pv. *syringae* Harpin_{PS}: a Protein that is Secreted via the Hrp

Pathway and Elicits the Hypersensitive Response in Plants," *Cell* 73:1255-1266

(1993), which is hereby incorporated by reference. The DNA molecule encoding the

hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide

sequence corresponding to SEQ. ID. No. 12 as follows:

15

ATGCAGAGTC TCAGTCTTAA CAGCAGCTCG CTGCAAACCC CGGCAATGGC CCTTGTCTCTG 60

GTACGTCCTG AAGCCGAGAC GACTGGCAGT ACGTCGAGCA AGGCGCTTCA GGAAGTTGTC 120

GTGAAGCTGG CCGAGGAACT GATGCGCAAT GGTCAACTCG ACGACAGCTC GCCATTGGGA 180

AAACTGTTGG CCAAGTCGAT GGCCGCAGAT GGCAAGGCGG GCGGCGGTAT TGAGGATGTC 240

20

ATCGCTGCGC TGGACAAGCT GATCCATGAA AAGCTCGGTG ACAACTTCGG CGCGTCTGCG 300

GACAGCGCCT CGGGTACCGG ACAGCAGGAC CTGATGACTC AGGTGCTCAA TGGCCTGGCC 360

AAGTCGATGC TCGATGATCT TCTGACCAAG CAGGATGGCG GGACAAGCTT CTCCGAAGAC 420

GATATGCCGA TGCTGAACAA GATCGCGCAG TTCATGGATG ACAATCCCGC ACAGTTTCCC 480

AAGCCGGACT CGGGCTCCTG GGTGAACGAA CTCAAGGAAG ACAACTTCCT TGATGGCGAC 540

25

GAAACGGCTG CGTTCCGTTC GGCACCTGAC ATCATTGGCC AGCAACTGGG TAATCAGCAG 600

AGTGACGCTG GCAGTCTGGC AGGGACGGGT GGAGGTCTGG GCACTCCGAG CAGTTTTTTC 660

AACAACCTCGT CCGTGATGGG TGATCCGCTG ATCGACGCCA ATACCGGTCC CGGTGACAGC 720

GGCAATACCC GTGGTGAAGC GGGGCAACTG ATCGGCGAGC TTATCGACCG TGGCCTGCAA 780

TCGGTATTGG CCGTGGTGG ACTGGGCACA CCCGTAAACA CCCCAGAGAC CGGTACGTCG 840

30

GCGAATGGCG GACAGTCCGC TCAGGATCTT GATCAGTTGC TGGGCGGCTT GCTGCTCAAG 900

GGCCTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT 960

GCGCAAATCG CCACCTTGCT GGTCAGTACG CTGCTGCAAG GCACCCGCAA TCAGGCTGCA 1020

GCCTGA 1026

Another potentially suitable hypersensitive response elicitor from *Pseudomonas syringae* is disclosed in U.S. Patent Application Serial No. 09/120,817, which is hereby incorporated by reference. The protein has a nucleotide sequence of SEQ. ID. No. 13 as follows:

	TCCACTTCGC	TGATTTTGAA	ATTGGCAGAT	TCATAGAAAC	G TTCAGGTGT	GGAAATCAGG	60
	CTGAGTGCGC	AGATTTCGTT	GATAAGGGTG	TGGTACTGGT	CATTGTTGGT	CATTTC AAGG	120
10	CCTCTGAGTG	CGGTGCGGAG	CAATACCAGT	CTTCCTGCTG	GCGTGTGCAC	ACTGAGT CGC	180
	AGGCATAGGC	ATTTCA GTTC	CTTGCGTTGG	TTGGGCATAT	AAAAAAAGGA	ACTTTTAAAA	240
15	ACAGTGCAAT	GAGATGCCGG	CAAAACGGGA	ACCGGTCGCT	GCGCTTTGCC	ACTCACTTCG	300
	AGCAAGCTCA	ACCCCAAACA	TCCACATCCC	TATCGAACGG	ACAGCGATAC	GGCCACTTGC	360
	TCTGGTAAAC	CCTGGAGCTG	GCGTCGGTCC	AATTGCCCCAC	TTAGCGAGGT	AACGCAGCAT	420
20	GAGCATCGGC	ATCACACCCC	GGCCGCAACA	GACCACCACG	CCACTCGATT	TTTCGGCGCT	480
	AAGCGGCAAG	AGTCCTCAAC	CAAACACGTT	CGGCGAGCAG	AACACTCAGC	AAGCGATCGA	540
25	CCCGAGTGCA	CTGTTGTTCC	GCAGCGACAC	ACAGAAAGAC	GTCAACTTCG	GCACGCCCCGA	600
	CAGCACCGTC	CAGAATCCGC	AGGACGCCAG	CAAGCCCAAC	GACAGCCAGT	CCAACATCGC	660
	TAAATTGATC	AGTGCA TTGA	TCATGTCGTT	GCTGCAGATG	CTCACC AACT	CCAATAAAAA	720
30	GCAGGACACC	AATCAGGAAC	AGCCTGATAG	CCAGGCTCCT	TTCCAGAACA	ACGGCGGGCT	780
	CGGTACACCG	TCGGCCGATA	GCGGGGGCGG	CGGTACACCG	GATGCGACAG	GTGGCGGGCGG	840
35	CGGTGATACG	CCAAGCGCAA	CAGGCGGTGG	CGGCGGTGAT	ACTCCGACCG	CAACAGGCGG	900
	TGGCGGCAGC	GGTGGCGGCG	GCACACCCAC	TGCAACAGGT	GGCGGCAGCG	GTGGCACACC	960
	CACTGCAACA	GGCGGTGGCG	AGGGTGGCGT	AACACCGCAA	ATCACTCCGC	AGTTGGCCAA	1020
40	CCCTAACCGT	ACCTCAGGTA	CTGGCTCGGT	GTCGGACACC	GCAGGTTCTA	CCGAGCAAGC	1080
	CGGCAAGATC	AATGTGGTGA	AAGACACCAT	CAAGGTCGGC	GCTGGCGAAG	TCTTTGACGG	1140
45	CCACGGCGCA	ACCTTCACTG	CCGACAAATC	TATGGGTAAC	GGAGACCAGG	GCGAAAATCA	1200
	GAAGCCCATG	TTGAGCTGG	CTGAAGGCGC	TACGTTGAAG	AATGTGAACC	TGGGTGAGAA	1260
	CGAGGTCGAT	GGCATCCACG	TGAAAGCCAA	AAACGCTCAG	GAAGTCACCA	TTGACAACGT	1320
50	GCATGCCCAG	AACGTCGGTG	AAGACCTGAT	TACGGTCAAA	GGCGAGGGAG	GCGCAGCGGT	1380
	CACTAATCTG	AACATCAAGA	ACAGCAGTGC	CAAAGGTGCA	GACGACAAGG	TTGTCCAGCT	1440
55	CAACGCCAAC	ACTCACTTGA	AAATCGACAA	CTTCAAGGCC	GACGATTTCG	GCACGATGGT	1500
	TCGCACCAAC	GGTGGCAAGC	AGTTTGATGA	CATGAGCATC	GAGCTGAACG	GCATCGAAGC	1560
60	TAACCACGGC	AAGTTCGCCC	TGGTGAAAAG	CGACAGTGAC	GATCTGAAGC	TGGCAACGGG	1620

- 25 -

CAACATCGCC ATGACCGACG TCAAACACGC CTACGATAAA ACCCAGGCAT CGACCCAACA 1680
 CACCGAGCTT TGAATCCAGA CAAGTAGCTT GAAAAAAGGG GGTGGACTC 1729

5

This DNA molecule is known as the dspE gene for *Pseudomonas syringae*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 14 as follows:

10

Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu
 1 5 10 15

15

Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly
 20 25 30

Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly
 35 40 45

20

Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val
 50 55 60

Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile
 65 70 75 80

25

Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr
 85 90 95

Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln
 100 105 110

30

Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser
 115 120 125

35

Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Gly Asp Thr
 130 135 140

Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly
 145 150 155 160

40

Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly
 165 170 175

Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr
 180 185 190

45

Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr
 195 200 205

50

Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile
 210 215 220

Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp
 225 230 235 240

55

- 26 -

[illegible]

This protein or polypeptide is about 42.9 kDa.

40 The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ. ID. No. 15 as follows:

45	Met	Ser	Val	Gly	Asn	Ile	Gln	Ser	Pro	Ser	Asn	Leu	Pro	Gly	Leu	Gln
	1				5					10					15	
	Asn	Leu	Asn	Leu	Asn	Thr	Asn	Thr	Asn	Ser	Gln	Gln	Ser	Gly	Gln	Ser
				20					25					30		
	Val	Gln	Asp	Leu	Ile	Lys	Gln	Val	Glu	Lys	Asp	Ile	Leu	Asn	Ile	Ile
			35					40					45			
50	Ala	Ala	Leu	Val	Gln	Lys	Ala	Ala	Gln	Ser	Ala	Gly	Gly	Asn	Thr	Gly
	50						55					60				

- 27 -

	Asn	Thr	Gly	Asn	Ala	Pro	Ala	Lys	Asp	Gly	Asn	Ala	Asn	Ala	Gly	Ala	
	65					70					75					80	
	Asn	Asp	Pro	Ser	Lys	Asn	Asp	Pro	Ser	Lys	Ser	Gln	Ala	Pro	Gln	Ser	
					85					90					95		
5	Ala	Asn	Lys	Thr	Gly	Asn	Val	Asp	Asp	Ala	Asn	Asn	Gln	Asp	Pro	Met	
					100					105				110			
	Gln	Ala	Leu	Met	Gln	Leu	Leu	Glu	Asp	Leu	Val	Lys	Leu	Leu	Lys	Ala	
					115					120				125			
10	Ala	Leu	His	Met	Gln	Gln	Pro	Gly	Gly	Asn	Asp	Lys	Gly	Asn	Gly	Val	
		130					135					140					
	Gly	Gly	Ala	Asn	Gly	Ala	Lys	Gly	Ala	Gly	Gly	Gln	Gly	Gly	Leu	Ala	
		145					150					155				160	
	Glu	Ala	Leu	Gln	Glu	Ile	Glu	Gln	Ile	Leu	Ala	Gln	Leu	Gly	Gly	Gly	
					165					170					175		
15	Gly	Ala	Gly	Ala	Gly	Gly	Ala	Gly	Gly	Gly	Val	Gly	Gly	Ala	Gly	Gly	
					180					185				190			
	Ala	Asp	Gly	Gly	Ser	Gly	Ala	Gly	Gly	Ala	Gly	Gly	Ala	Asn	Gly	Ala	
					195					200				205			
20	Asp	Gly	Gly	Asn	Gly	Val	Asn	Gly	Asn	Gln	Ala	Asn	Gly	Pro	Gln	Asn	
		210					215						220				
	Ala	Gly	Asp	Val	Asn	Gly	Ala	Asn	Gly	Ala	Asp	Asp	Gly	Ser	Glu	Asp	
		225					230				235					240	
	Gln	Gly	Gly	Leu	Thr	Gly	Val	Leu	Gln	Lys	Leu	Met	Lys	Ile	Leu	Asn	
					245					250					255		
25	Ala	Leu	Val	Gln	Met	Met	Gln	Gln	Gly	Gly	Leu	Gly	Gly	Gly	Asn	Gln	
					260					265					270		
	Ala	Gln	Gly	Gly	Ser	Lys	Gly	Ala	Gly	Asn	Ala	Ser	Pro	Ala	Ser	Gly	
					275					280				285			
30	Ala	Asn	Pro	Gly	Ala	Asn	Gln	Pro	Gly	Ser	Ala	Asp	Asp	Gln	Ser	Ser	
		290					295					300					
	Gly	Gln	Asn	Asn	Leu	Gln	Ser	Gln	Ile	Met	Asp	Val	Val	Lys	Glu	Val	
		305					310				315				320		
	Val	Gln	Ile	Leu	Gln	Gln	Met	Leu	Ala	Ala	Gln	Asn	Gly	Gly	Ser	Gln	
					325					330					335		
35	Gln	Ser	Thr	Ser	Thr	Gln	Pro	Met									
							340										

It is encoded by a DNA molecule having a nucleotide sequence corresponding SEQ.
ID. No. 16 as follows:

- 28 -

ATGTCAGTCG GAAACATCCA GAGCCCGTCG AACCTCCCGG GTCTGCAGAA CCTGAACCTC 60
 AACACCAACA CCAACAGCCA GCAATCGGGC CAGTCCGTGC AAGACCTGAT CAAGCAGGTC 120
 GAGAAGGACA TCCTCAACAT CATCGCAGCC CTCGTGCAGA AGGCCGCACA GTCGGCGGGC 180
 GGCAACACCG GTAACACCGG CAACGCGCCG GCGAAGGACG GCAATGCCAA CGCGGGCGCC 240
 5 AACGACCCGA GCAAGAACGA CCCGAGCAAG AGCCAGGCTC CGCAGTCGGC CAACAAGACC 300
 GGCAACGTCG ACGACGCCAA CAACCAGGAT CCGATGCAAG CGCTGATGCA GCTGCTGGAA 360
 GACCTGGTGA AGCTGCTGAA GGCGGCCCTG CACATGCAGC AGCCCGGCGG CAATGACAAG 420
 GGCAACGGCG TGGGCGGTGC CAACGGCGCC AAGGGTGCCG GCGGCCAGGG CGGCCTGGCC 480
 GAAGCGCTGC AGGAGATCGA GCAGATCCTC GCCCAGCTCG GCGGCGGCGG TGCTGGCGCC 540
 10 GGCGGCGCGG GTGGCGGTGT CGGCGGTGCT GGTGGCGCGG ATGGCGGCTC CGGTGCGGGT 600
 GGCGCAGGCG GTGCGAACGG CGCCGACGGC GGCAATGGCG TGAACGGCAA CCAGGCGAAC 660
 GGCCCGCAGA ACGCAGGCGA TGTCAACGGT GCCAACGGCG CGGATGACGG CAGCGAAGAC 720
 CAGGGCGGCC TCACCGGCGT GCTGCAAAAG CTGATGAAGA TCCTGAACGC GCTGGTGCAG 780
 ATGATGCAGC AAGCGGCCTT CGGCGGCGGC AACCAGGCGC AGGGCGGCTC GAAGGGTGCC 840
 15 GGCAACGCCT CGCCGGCTTC CGGCGCGAAC CCGGGCGCGA ACCAGCCCGG TTCGGCGGAT 900
 GATCAATCGT CCGGCCAGAA CAATCTGCAA TCCCAGATCA TGGATGTGGT GAAGGAGGTC 960
 GTCCAGATCC TGCAGCAGAT GCTGGCGGCG CAGAACGGCG GCAGCCAGCA GTCCACCTCG 1020
 ACGCAGCCGA TGTA 1035

20 Further information regarding the hypersensitive response elicitor polypeptide or
 protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van
 Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which
 Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted
 25 via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994),
 which is hereby incorporated by reference.

The hypersensitive response elicitor polypeptide or protein from
Xanthomonas campestris pv. glycines has an amino acid sequence corresponding to
 SEQ. ID. No. 17 as follows:

30

Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala
 1 5 10 15

- 29 -

Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
20 25

5 This sequence is an amino terminal sequence having only 26 residues from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris* pv. glycines. It matches with fimbrial subunit proteins determined in other *Xanthomonas campestris* pathovars.

The hypersensitive response elicitor polypeptide or protein from
10 *Xanthomonas campestris* pv. *pelargonii* is heat stable, protease sensitive, and has a molecular weight of 20 kDa. It includes an amino acid sequence corresponding to SEQ. ID. No. 18 as follows:

15 Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
1 5 10 15
Leu Leu Ala Met
20

20 Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui et al., "The RsmA Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrp* N_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or
25 polypeptide of *Erwinia stewartii* is set forth in Ahmad et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

30 Hypersensitive response elicitor proteins or polypeptides from *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamoni*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora* are described in Kaman, et al., "Extracellular Protein Elicitors from *Phytophthora*: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens,"
35 Molec. Plant-Microbe Interact., 6(1):15-25 (1993), Ricci et al., "Structure and Activity of Proteins from Pathogenic Fungi *Phytophthora* Eliciting Necrosis and

Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989), Ricci et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of *Phytophthora parasitica*," Plant Path. 41:298-307 (1992), Baillreul et al, "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference.

Another hypersensitive response elicitor in accordance with the present invention is from *Clavibacter michiganensis* subsp. *sepedonicus* which is fully described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference.

The above elicitors are exemplary. Other elicitors can be identified by growing fungi or bacteria that elicit a hypersensitive response under conditions which genes encoding an elicitor are expressed. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

Fragments of the above hypersensitive response elicitor polypeptides or proteins as well as fragments of full length elicitors from other pathogens are encompassed by the method of the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for
5 expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by
10 conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of a hypersensitive response elicitor which do not elicit a hypersensitive response include fragments of the *Erwinia*. Suitable fragments include a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, or
15 an internal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 169 and 403, 210 and 403, 267 and 403, or 343 and 403. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 105 and 179, 137 and 166, 121 and 150, or
20 137 and 156. Other suitable fragments can be identified in accordance with the present invention.

Another example of suitable fragments of a hypersensitive response elicitor which do elicit a hypersensitive response are *Erwinia amylovora* fragments including a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N-
25 terminal fragment of the amino acid sequence of SEQ. ID. No. 3, or an internal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span amino acids 105 and 403 of SEQ. ID. No. 3. The N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 1 and 98, 1 and 104, 1
30 and 122, 1 and 168, 1 and 218, 1 and 266, 1 and 342, 1 and 321, and 1 and 372. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the

following amino acids of SEQ. ID. No. 3: 76 and 209, 105 and 209, 99 and 209, 137 and 204, 137 and 200, 109 and 204, 109 and 200, 137 and 180, and 105 and 180.

Suitable DNA molecules are those that hybridize to the DNA molecule comprising a nucleotide sequence of SEQ. ID. Nos. 2, 4, 5, 7, 9, 12, 13, and 16 under
5 stringent conditions. An example of suitable high stringency conditions is when hybridization is carried out at 65°C for 20 hours in a medium containing 1M NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.1% sodium dodecyl sulfate, 0.2% ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 50 µm g/ml *E. coli* DNA.

Variants may be made by, for example, the deletion or addition of
10 amino acids that have minimal influence on the properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis,
15 purification, or identification of the polypeptide.

The hypersensitive response elicitor of the present invention is preferably in isolated form (i.e. separated from its host organism) and more preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional techniques. Typically, the hypersensitive response elicitor of the
20 present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the protein or polypeptide of the present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove
25 bacterial debris. The supernatant is then subjected to heat treatment and the hypersensitive response elicitor is separated by centrifugation. The supernatant fraction containing the hypersensitive response elicitor is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the fragment. If necessary, the protein fraction may be further purified by ion exchange or HPLC.

30 The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an

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expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccina virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA;

microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promotors differ from those of procaryotic promotors. Furthermore, eucaryotic promotors and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, procaryotic promotors are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promotors vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promotors in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promotors may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promotors such as the T7 phage promotor, *lac* promotor,

trp promotor, *recA* promotor, ribosomal RNA promotor, the P_R and P_L promotors of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5 (tac)* promotor or other *E. coli* 5 promotors produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promotor unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted 10 DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation 15 initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promotor, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to 20 provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving 25 incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell 30 system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

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The present invention's method of imparting stress resistance to plants can involve applying the hypersensitive response elicitor polypeptide or protein in a non-infectious form to all or part of a plant or a plant seed under conditions effective for the elicitor to impart stress resistance. Alternatively, the hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart stress resistance in plants.

As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart stress resistance in plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the plant under conditions effective to permit that DNA molecule to impart stress resistance to plants. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to impart stress resistance to plants.

The embodiment of the present invention where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated hypersensitive response elicitor or 2) application of bacteria which do not cause disease and are transformed with a genes encoding the elicitor. In the latter embodiment, the elicitor can be applied to plants or plant seeds by applying bacteria containing the DNA molecule encoding a hypersensitive response elicitor polypeptide or protein. Such bacteria must be capable of secreting or exporting the elicitor so that the elicitor can contact plant or plant seed cells. In these embodiments, the elicitor is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

The methods of the present invention can be utilized to treat a wide variety of plants or their seeds to impart stress resistance. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea,

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chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

In accordance with the present invention, the term "stress" refers to drought, salt, cold temperatures (e.g., frost), chemical treatment (e.g., insecticides, fungicides, herbicides, fertilizers), water, excessive light, and insufficient light.

10 The method of the present invention involving application of the hypersensitive response elicitor polypeptide or protein can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, propagules (e.g., cuttings), etc. This may (but need not) involve infiltration of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds or propagules (e.g., cuttings), in accordance with the application embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide, in accordance with present invention, can be applied by low or high pressure spraying, coating, immersion, or injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the elicitor with cells of the plant or plant seed. Once treated with the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the hypersensitive response elicitor protein or polypeptide to impart stress resistance to plants.

30 The hypersensitive response elicitor polypeptide or protein, in accordance with the present invention, can be applied to plants or plant seeds alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor

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polypeptide or protein can be applied separately to plants with other materials being applied at different times.

A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a hypersensitive
5 response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains greater than 500 nM of the elicitor.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematocide, and mixtures thereof.
10 Suitable fertilizers include $(\text{NH}_4)_2\text{NO}_3$. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response elicitor can
15 be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, a hypersensitive response elicitor need not be applied topically to the plants or seeds. Instead, transgenic plants transformed
20 with a DNA molecule encoding such an elicitor are produced according to procedures well known in the art.

The vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by
25 reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby incorporated by reference.

Another approach to transforming plant cells with a gene is particle bombardment (also known as biolistic transformation) of the host cell. This can be
30 accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby

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incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

Agrobacterium is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a

convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by Agrobacterium and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedure with the presence of the

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gene encoding the hypersensitive response elicitor resulting in stress resistance to the plant. Alternatively, transgenic seeds or propagules (e.g., cuttings) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions effective to impart stress resistance to plants. While not wishing to be bound by theory, such stress resistance may be RNA mediated or may result from expression of the elicitor polypeptide or protein.

When transgenic plants and plant seeds are used in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a hypersensitive response elicitor in accordance with the present invention is applied. These other materials, including a hypersensitive response elicitor in accordance with the present invention, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, and immersion. Similarly, after plants have been propagated from the transgenic plant seeds, the plants may be treated with one or more applications of the hypersensitive response elicitor in accordance with the present invention to impart stress resistance. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.).

EXAMPLES

Example 1 - Hypersensitive Response Elicitor-Treated Cotton is More Resistant to the Damage Caused by Insecticide Stress

Aphids (*Aphis gossypii*) infect cotton during the entire growth season. The damage of aphid infection ranges from honeydew deposit that contaminates the lint and reduces crop value to defoliation that reduces or destroys crops. To protect plants from aphid infection, cotton is usually sprayed with insecticides, for example Asana XL when the infection pressure is not very high, and Admire when the infestation pressure is high. The effect of a hypersensitive response elicitor on aphids in cotton was studied by a trial involving a randomized complete block design. This

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involved treatment with *Erwinia amylovora* hypersensitive response elicitor (i.e. HP-1000™) at 20, 60, and 80 ppm and a chemical insecticide, Asana XL, at 8 oz./ac. Each treatment involved foliar application beginning at cotyledon to three true leaves and thereafter at 14 day intervals using a backpack sprayer. Aphid counts and overall growth of the cotton were made immediately prior to spray application at 14, 28, 35, and 42 days after the first treatment ("DAT 1"). Twenty-five randomly selected leaves per plot were collected at the first three sampling dates and the leaves per plot at the final sampling date.

10. Results

1. Aphid control: The number of aphids in the hypersensitive response elicitor-treated cotton were significantly reduced in comparison to the chemical treated cotton (see Table 1).

15 Table 1. Aphid count per leaf on cotton after treatment with Asana XL® or HP-1000™

Treatment	Rate ²	Number of aphids per leaf ¹			
		No. sprays applied/days after treatment			
		1/14DAT1	2/28DAT1	3/35DAT1	4/42DAT1
Asana XL®	8 oz/ac	0.2 a	32.2 a	110.0 a	546.9 a
HP-1000™	20 µg/ml	0.2 a	7.8 b	22.9 b	322.1 a
HP-1000™	60 µg/ml	0.1 a	4.9 b	34.6 b	168.3 a
HP-1000™	80 µg/ml	0.0 a	2.7 b	25.8 b	510.2 a

¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05. ²Rate for Asana XL® is for formulated product, rate for HP-1000™ is for active ingredient (a.i.).

At 14 days after DAT 1, aphid counts were relatively low across all of the treatments, but by 28 days after DAT 1 (by which time two sprayings had been applied), the number of aphids per leaf were significantly greater in Asana XL-treated plants compared to the hypersensitive response elicitor-treated cottons. By 35 days after DAT 1 (by which time three sprayings had been applied), aphid counts had risen for all treatments, yet aphid counts per leaf were still significantly lower for hypersensitive response elicitor-treated cotton compared to the Asana XL treatment. Finally, at 42 days after DAT 1 (by which time four sprayings had been applied), the number of aphids per leaf had increased to a level that threatened to overwhelm the

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plants even when treated with the standard chemical insecticide. To save the trial, another chemical, Pravado (Admire), was applied to all plots to eradicate aphids from the field.

2. Hypersensitive response elicitor-treated cotton was more resistant to the damage caused by Pravado (Admire) and Asana. After the second chemical spraying, it was observed that cotton plants were stress shocked by the insecticides. The cotton plants previously treated with Asana and untreated control were defoliated. On most of the chemical-treated cotton, there were no leaves, or very few leaves, in the lower portion of plants. However, the hypersensitive response elicitor-treated plants, especially the plot where hypersensitive response elicitor was applied at 80 ppm, had no defoliation and the cotton plants were vigorous and healthy. By counting the number of mature balls, it clearly showed that hypersensitive response elicitor-treated plants (at 80 ppm) had more ball setting than chemical and untreated control (Table 2), indicating that hypersensitive response elicitor-treated plants were more tolerant to the stress caused by insecticide.

Table 2. Number of Formed Cotton Balls Counted on Ten Plants in Each of Four Replicates Per Treatment.

<u>Treatment</u>	<u>No. balls/10 plants/replicate</u>
UTC	28
Chemical standard	6
Hypersensitive Response Elicitor	35

Example 2 - Hypersensitive Response Elicitor-Treated Cucumbers are More Resistant to Drought

- A cucumber field trial was set up to test the effect of *Erwinia amylovora* hypersensitive response elicitor on disease control, tolerance to drought stress, and yield. Three different rates were tested, there at 15, 30, and 60 µg/ml. In addition to hypersensitive response elicitor treatment, there was an untreated control. Each treatment contained three replicate plots. When the first true leaf emerges, hypersensitive response elicitor was sprayed with a back bag sprayer. The second spray was applied ten days after the first spray. The third application was right after

the recovery of cucumber seedlings after the transplanting to the field. Individual treatment was randomly assigned in the field.

When the first true leaf emerged (Day 0), a first application was sprayed. Usually cucumber seedlings are transplanted when seedlings show two true
5 leaves. It has been known that the recovery rate after the transplanting is closely related to the size of the seedlings. Because of the drought, the seedlings were maintained in the nursery for an extra ten days and the second spray was applied on Day 10. Two days after the second spray, the plants were transplanted into fields and covered with plastic sheets. The plants had 4 – 5 true leaves.

10

Result

The recovery rate of the transplanted cucumber seedlings was higher for the hypersensitive response elicitor-treated plants than for the untreated control. More than 80% of the hypersensitive response elicitor-treated cucumber seedlings
15 survived, while only 57% untreated plants survived.

Throughout the growth season, there was a serious drought problem. Early field visits indicated that hypersensitive response elicitor-treated plants had more root mass and better over-all growth. Hypersensitive response elicitor-treated cucumber started to flower 14 days earlier than untreated control cucumber. The
20 early flowering resulted in an earlier harvest. In the first harvest, more than 0.4 kilograms of cucumber fruits per plant were harvested from the hypersensitive response elicitor-treated cucumbers; however, virtually no fruit was harvested from untreated control. By the end of the season, untreated plants died due to severe drought, but hypersensitive response elicitor-treated plants were still alive and had
25 one more harvest.

The final yield was significantly different between hypersensitive response elicitor-treated and untreated plants. Hypersensitive response elicitor administered at the rate of 30 ppm produced three times greater yield than the control plants (Table 3).

30

Table 3. Yield Increase of Cucumber Fruit from Hypersensitive Response Elicitor Treated Plants

Treatment	Replicate	kg/plant	Yield/Replicate	% of the Yield Increase
HP 15	I	1.25	37.5	
	II	1.00	30.0	103.8
	III	1.21	36.3	241
HP 30	I	1.54	46.2	
	II	1.43	42.9	133.2
	III	1.47	44.1	339
Control	I	0.43	12.9	
	II	0.41	12.3	39.3
	III	0.47	14.1	

5

The increased yield was partially attributed to hypersensitive response elicitor-induced growth enhancement and partially resulted from more tolerance of hypersensitive response elicitor-treated cucumber to drought, because usually the yield increase from hypersensitive response elicitor-induced growth enhancement is between 10-40%.

10

Example 3 - Hypersensitive Response Elicitor-Treated Pepper is More Tolerant to Herbicide Stress

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Pepper seedlings were drenched with hypersensitive response elicitor at 20 ppm seven days before transplanting, sprayed seven days after the transplanting, and then, sprayed every fourteen days. Standard chemicals, Brave, Maneb, Kocide, and Admire, were used for the rest of the treatment. In addition to early growth enhancement, which resulted in a higher yield, larger fruit, and resistance to several diseases, hypersensitive response elicitor-treated pepper was more tolerant to herbicide damage. The pepper field was applied with the herbicide SENCOR which is not labeled for pepper. This herbicide is known to cause severe foliar damage to pepper in chemically-treated plants but not with hypersensitive response elicitor-treated plants.

20

25

The difference between the adverse effect of the herbicide on the hypersensitive response elicitor and non-hypersensitive response elicitor treated plants is dramatic. See Table 4 below. Thirty-nine of the 60 elicitor-treated plants showed only minor damage by the herbicide, the damaged leaves were less than 20%. In

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contrast, 53 out of the 60 chemically-treated pepper plants had severe damage, 40-57% of the leaves were damaged, and 20 plants were dead. The ability of hypersensitive response elicitors to help crops withstand the phytotoxic effects of a herbicide is very important benefit to in agricultural industry.

5

Table 4. Hypersensitive Response Elicitor-Treated Peppers are More Tolerant to Herbicide Damage.

10	<u>Treatment</u>	<u>Damage Rating</u>						<u>Damage Index %</u>
		1	2	3	4	5	6	
	Hypersensitive Response Elicitor	1	38	17	3	1	0	41
15	Chemicals	0	1	6	16	19	18	87

Damage Rating: 1. No damage; 2. 0-20% leaves damaged; 3. 20-40% leaves damaged; 4. 40-50% leaves damaged; 6. More than 75% leaves damaged or entire plant dead.

20

Damage index = sum of each rating times the number of plants under the rating scale, divided by total number of plants times 6.

Damage index for hypersensitive response elicitor-treated plants = $\frac{1 \times 1 + 2 \times 38 + 3 \times 17 + 4 \times 3 + 5 \times 1 + 6 \times 0}{6 \times 60} \times 100\% = 41\%$

25

Example 4 - Hypersensitive Response Elicitor-Treated Pepper is More Tolerant to Herbicide Stress under Controlled Experimental Conditions

30

A field trial was conducted to test if hypersensitive elicitor treated pepper would be more tolerant to herbicide stress. The trial contains 6 treatments and 4 replicates for each treatment. The treatments are described as follows:

1. Control, the peppers were neither treated by a hypersensitive response ("HR") elicitor nor by LEXONE™ herbicide (DuPont Agricultural Products, Wilmington, Delaware).
2. Control pepper with application of 0.15 pound LEXONE™ herbicide /acre.
3. Control pepper with application of 0.3 pound LEXONE™ herbicide /acre.

40

4. HR elicitor treatment with no application of LEXONE™ herbicide using a formulated product known as MESSENGER™ biopesticide (Eden Bioscience Corporation, Bothell, Washington) containing 3% HR elicitor protein was used.

5 5. HR elicitor treatment with application of 0.15 pound LEXONE™ herbicide /acre.

6. HR elicitor treatment with application of 0.3 pound LEXONE™ herbicide /acre.

LEXONE™ contains the same active ingredient as SENCOR™ herbicide (Bayer, Kansas City, Missouri) used in Example 3. Pepper seedlings were
10 drenched with MESSENGER™ solution at the concentration of HR elicitor protein of about 20 ppm seven days before transplanting into the field and then sprayed every 14 days after the transplanting. LEXONE was applied at high (0.3 pound/acre) and low levels (0.15 pound/acre). 50 gallon water and 100 mL of the herbicide solution was
15 introduced into the root zone of each plant in the respective treatment five weeks after transplant into the field.

The treatments were evaluated for the percent of chlorosis caused by the LEXONE™ herbicide application and for the pepper yield. HR elicitor-treated plants exposed to the high rate of herbicide had significantly less chlorosis and
20 produced 108 % more fruit in comparison to the non-hypersensitive response elicitor treated plants exposed to the same amount of herbicide. See Tables 5 and 6 below. There was no significant difference in the reduction of chlorosis at the low rate of herbicide between the HR elicitor treated and non-HR elicitor treated peppers. However, the HR elicitor treated plants produced 15% more fruit than the
25 corresponding control plants exposed to the same amount of herbicide. There was no chlorosis in either the check or HR elicitor-treated plants that did not receive LEXONE™ herbicide treatment.

The HR elicitor treated plants were much less severely affected by the herbicide application than the respective control plants at the high rate of herbicide.
30 However, the amount of visual chlorosis was similar at the low rate for both the check and HR elicitor-treated plants. More importantly, the yields from both the high and low rate herbicide treatments of HR elicitor treated plants were less severely effected

by the herbicide than the checks. These findings further confirm that HR elicitors can help crops withstand the phytotoxic effects of herbicides and are very beneficial to the agricultural industry.

5 Table 5. Reduction of Foliar Chlorosis and Increase in Yield in Hypersensitive Response Elicitor Treated Plants after Exposure to LEXONE™ Herbicide

Treatment	Percent foliar chlorosis and yield of pepper						
	A	B	C	D	E	Yield (pound)	% difference from the respective control
6 (MESSENGER™ + High rate LEXONE™)	13.75	30.00	37.50	36.25	40.00	8.31	108 %
3 (High rate LEXONE™)	26.25	43.75	51.25	50.00	51.25	4.00	-
5 (MESSENGER™ + low rate LEXONE™)	16.25	22.50	28.75	23.75	27.50	8.00	15 %
2 (LENOXE™)	12.50	20.00	25.00	25.00	23.75	6.81	-

10 Table 6. Weight of Harvested Peppers Increased in Hypersensitive Response Elicitor Treated Plants after Exposure to LEXONE™ Herbicide Compared to Check Plants.

Treatment	Weight of peppers harvested 12/1/98 in pounds
HP20 + high rate LEXONE™	8.31
Check + high rate LEXONE™	4.00
HP20 + low rate LEXONE™	8.00
Check + low rate LEXONE™	6.81

15 **Example 5 - Hypersensitive Response Elicitor-Treated Cotton is More Tolerant to Drought Stress**

A non-irrigated cotton trial experienced 26 consecutive days of drought. The average daily heat index was near or over 100 degrees F, adding to the stress placed on the plants in the field.

20

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Observations in the field indicated that plants treated with HR elicitor at the concentration of 15 ppm (2.2 oz formulated product, MESSENGER™ containing 3 % active ingredient HR elicitor protein) were more vigorous and had less defoliation than the check plants as a result of the heat and drought stress. Equal numbers of plants from the MESSENGER™-treated and the non-MESSENGER™ treated plots were carefully removed from the field and mapped for the number of nodes and bolls by position. The plants were also weighed on a Metler analytical scale to determine whole plant, root and shoot weights.

MESSENGER™ treated plants survived the heat and drought stresses much better than the untreated plants did. Plants treated with MESSENGER™ had 37.6% more root and shoot mass than the check plants (Table 7). The MESSENGER™ treated plants also had significantly more cotton bolls than the check plants (Table 8). The number of cotton bolls from positions 1 and 2 have a significant contribution to the overall yield. Table 8 showed that MESSENGER™ treated plants had 47% more bolls in positions 1 and 2 and 57% more boll from a whole plant in comparison to the yield achieved using a grower standard treatment (i.e. with no MESSENGER™ treatment). A common reaction to stress in cotton is for the plant to abort bolls. The results indicate that MESSENGER™-treated plants are more tolerant to the drought stress.

20

Table 7. Weight per Plant of Non-Irrigated Cotton Following 26 Days of Drought.

Treatment	Root weight (pond/plant)	%Difference	Shoot weight (pond/plant)	% difference	Whole plant weight (pond/plant)	% difference
MESSENGER™ 2.2 oz/acre	0.041 a*	37.6 %	0.505 a	37.5 %	0.546	37.5 %
Control (Grower standard)	0.0298 b		0.367 b		0.397	
Level of statistically significant	P=0.119		P=0.034			P=0.033

25

* Same letter indicates no statistical difference between the two treatments at the defined level; different letter indicates a statistical difference between the two treatments at the defined level.

Table 8. Number of Bolls per 5 Plants at the Number 1 & 2 positions, and Total Number of Bolls from Whole Plants in Non-irrigated Cotton Following 26 days of drought.

5

Treatment	Avg. # bolls in the #1 & 2 position	Percent difference	Avg. # of total bolls per 5 plant	Percent difference
MESSENGER™ 2.2 OZ.	18.4 a	+46.0%	21.4 a	+57.0%
Check	12.6 b		13.6 b	-
Statistically significant level	P=0.032		P=0.01	

* Same letter indicates no statistical difference between the two treatments at the defined level; different letter indicates a statistical difference between the two treatments at the defined level.

10 **Example 6 - Hypersensitive Response Elicitor-Treated Tomato is More Tolerant to Calcium Deficiency**

Calcium is an important element for plant physiology and development. A deficiency in calcium can cause several plant diseases. For example, blossom-end rot is caused by a localized calcium deficiency in the distal end of the tomato fruit. Because calcium is not a highly mobile element, a deficiency can occur with a fluctuation in water supply. In the past, tomato growers experienced higher level of blossom-end rot during dry weather conditions when infrequent rains storms dumped a lot of water and then return to a hot and dry condition quickly. Lowering or raising the irrigation water table erratically during a dry and hot growing season can also increase the disease.

A field trial was designed to test if HR elicitor protein-treated tomato can be more tolerant to the calcium deficiency under a dry hot growing season. MESSENGER™, the formulated product containing 3% HR elicitor, was used for the trial. The application rate of the MESSENGER™ was 2.27 oz per care. The first spray of MESSENGER™ was carried out 7 days before the transplanting and then every 14-days after transplanting. MESSENGER™-treated tomatoes were compared with a standard grower treatment not utilizing MESSENGER™. Each treatment had 4 replicates.

30 The number of infected fruit was counted from a 100 square foot field. The rot typically begins with light tan water soaked lesions, which then enlarge, and then turn black. In a survey, about 20% of the fruits were infected. Severe end-rot

symptoms occurred in the standard treatment; however, an average of only 2.5 % of the fruit was infected in the MESSENGER™-treated plants. The harvest data showed that MESSENGER™-treated plants had 8% more marketable fruit (Table 9). The test results demonstrated that MESSENGER™-treatment can reduce the stress resulting from calcium deficiency and increase plant resistance to blossom-end rot.

Table 9. Hypersensitive Response Elicitor Treatment Reduced Blossom-End Rot Infection, Increased Yield of Tomato Fruit

Treatment	Blossom-End Infected Fruit*				Tomato Fruit Yield	
	Rep I	Rep II	Rep III	Rep IV	Bin/Acre	% Difference
MESSENGER™	0	9	0	1	35	8
Standard Treatment)	24	22	16	17	31.5	-

*The data were collected from the fruits in 100 square foot plot

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

WHAT IS CLAIMED:

1. A method of imparting stress resistance to plants comprising:
applying a hypersensitive response elicitor protein or
5 polypeptide in a non-infectious form to a plant or plant seed under conditions
effective to impart stress resistance.
2. A method according to claim 1, wherein the stress resistance is
resistance to a stress selected from the group consisting of climated related stress, air
10 pollution stress, chemical stress, and nutritional stress.
3. A method according to claim 2, wherein the stress is chemical
stress where the chemical is selected from the group consisting of insecticides,
fungicides, herbicides, and heavy metals.
15
4. A method according to claim 2, wherein the stress is climate-
related stress selected from the group consisting of drought, water, frost, cold
temperature, high temperature, excessive light, and insufficient light.
- 20 5. A method according to claim 2, wherein the stress is air
pollution stress selected from the group consisting of carbon dioxide, carbon
monoxide, sulfur dioxide, NO_x, hydrocarbons, ozone, ultraviolet radiation, and acidic
rain.
- 25 6. A method according to claim 2, wherein the stress is nutritional
stress where the nutritional stress is caused by fertilizer, micronutrients, or
macronutrients.
7. A method according to claim 1, wherein the hypersensitive
30 response elicitor protein or polypeptide is derived from *Erwinia*, *Pseudomonas*,
Xanthamonas, *Phytophthora*, or *Clavibacter*.

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8. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*, *Erwinia carotovora*, *Erwinia chrysanthemi*, and *Erwinia stewartii*.
- 5 9. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas syringae* or *Pseudomonas solanacearum*.
- 10 10. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from a *Xanthomonas* species.
11. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from a *Phytophthora*.
- 15 12. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Clavibacter michiganensis* subsp. *sepedonicus*.
- 20 13. A method according to claim 1, wherein plants are treated during said applying.
14. A method according to claim 1, wherein plant seeds are treated during said applying, said method further comprising:
planting the seeds treated with the hypersensitive response
25 elicitor protein or polypeptide in natural or artificial soil and propagating plants from seeds planted in soil.
15. A method according to claim 1, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower,
30 peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear,

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melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

16. A method according to claim 1, wherein the plant is selected
5 from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

17. A method of imparting stress resistance to plants comprising:
providing a transgenic plant or plant seed transformed with a
10 DNA molecule which encodes for a hypersensitive response elicitor protein or polypeptide and
growing the transgenic plant or plants produced from the
transgenic plant seeds under conditions effective to impart stress resistance.

18. A method according to claim 17, wherein a transgenic plant is
15 provided.

19. A method according to claim 17, wherein a transgenic plant
seed is provided, said method further comprising:
20 planting the transgenic seeds in natural or artificial soil and
propagating plants from seeds planted in soil..

20. A method according to claim 17, wherein the stress resistance
is resistance to a stress selected from the group consisting of climated related stress,
25 air pollution stress, chemical stress, and nutritional stress.

21. A method according to claim 20, wherein the stress is chemical
stress where the chemical is selected from the group consisting of insecticides,
fungicides, herbicides, and heavy metals.

30

22. A method according to claim 20, wherein the stress is climate-related stress selected from the group consisting of drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light.

5 23. A method according to claim 20, wherein the stress is air pollution stress selected from the group consisting of carbon dioxide, carbon monoxide, sulfur dioxide, NO_x, hydrocarbons, ozone, ultraviolet radiation, and acidic rain.

10 24. A method according to claim 20, wherein the stress is nutritional stress where the nutritional stress is caused by fertilizer, micronutrients, or macronutrients.

15 25. A method according to claim 20, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Phytophthora*, or *Clavibacter*.

20 26. A method according to claim 25, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*, *Erwinia carotovora*, *Erwinia chrysanthemi*, and *Erwinia stewartii*.

25 27. A method according to claim 25, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas syringae* or *Pseudomonas solanacearum*.

28. A method according to claim 25, wherein the hypersensitive response elicitor protein or polypeptide is derived from a *Xanthomonas* species.

30 29. A method according to claim 20, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic,

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eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

- 5 30. A method according to claim 20, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

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<213> *Erwinia amylovora*

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<213> *Pseudomonas solanacearum*

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/26039

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/82 C12N15/31 A01N63/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 32844 A (CORNELL RES FOUNDATION INC) 30 July 1998 (1998-07-30) claims	1-30
A	WO 96 39802 A (CORNELL RES FOUNDATION INC) 19 December 1996 (1996-12-19) claims	1-30
A	WO 98 24297 A (CORNELL RES FOUNDATION INC) 11 June 1998 (1998-06-11) claims	1-30
A	WO 98 37752 A (CORNELL RES FOUNDATION INC) 3 September 1998 (1998-09-03) claims	1-30

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "A" document member of the same patent family

Date of the actual completion of the international search

22 May 2000

Date of mailing of the international search report

07/06/2000

Name and mailing address of the ISA

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Authorized officer

Decorte, D

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/26039

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9832844 A	30-07-1998	AU 6043198 A PL 334907 A	18-08-1998 27-03-2000
WO 9639802 A	19-12-1996	US 5650387 A AU 714512 B AU 5982196 A BR 9609073 A CA 2223616 A CN 1192647 A EP 0871354 A FI 974430 A JP 11506938 T NZ 309611 A PL 323823 A US 5859324 A US 5776889 A	22-07-1997 06-01-2000 30-12-1996 26-01-1999 19-12-1996 09-09-1998 21-10-1998 26-01-1998 22-06-1999 30-08-1999 27-04-1998 12-01-1999 07-07-1998
WO 9824297 A	11-06-1998	AU 5693598 A CN 1245393 A EP 0957672 A PL 334126 A	29-06-1998 23-02-2000 24-11-1999 14-02-2000
WO 9837752 A	03-09-1998	AU 6666498 A BR 9807632 A US 5977060 A	18-09-1998 22-02-2000 02-11-1999